

COUNTWAY LIBRARY



HC 4N2F U

BOSTON  
MEDICAL LIBRARY  
8 THE FENWAY











YALE UNIVERSITY  
MRS. HEPSA ELY SILLIMAN MEMORIAL LECTURES  
==  
THE ANATOMY AND PHYSIOLOGY  
OF CAPILLARIES





THE  
ANATOMY AND PHYSIOLOGY  
OF  
CAPILLARIES

✓ BY  
AUGUST KROGH, PH.D., LL.D.

PROFESSOR OF ZOÖ-PHYSIOLOGY  
COPENHAGEN UNIVERSITY

*Revised and Enlarged Edition.*



NEW HAVEN  
YALE UNIVERSITY PRESS  
LONDON • HUMPHREY MILFORD • OXFORD UNIVERSITY PRESS

COPYRIGHT, 1922, 1929, BY YALE UNIVERSITY PRESS

PRINTED IN THE UNITED STATES OF AMERICA

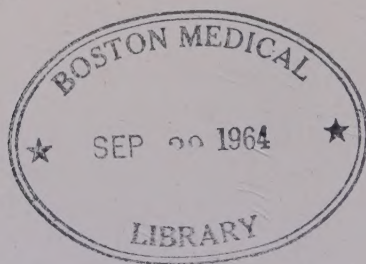
First Published, December, 1922

Second Printing, February, 1924

Revised and Enlarged Edition, June, 1929

Second Printing, August, 1930

All rights reserved. This book may not be reproduced, in whole or in part, in any form, except by written permission from the publishers.



1522

## THE SILLIMAN FOUNDATION

IN the year 1883 a legacy of eighty thousand dollars was left to the President and Fellows of Yale College in the city of New Haven, to be held in trust, as a gift from her children, in memory of their beloved and honored mother, Mrs. Hepsa Ely Silliman.

On this foundation Yale College was requested and directed to establish an annual course of lectures designed to illustrate the presence and providence, the wisdom and goodness of God, as manifested in the natural and moral world. These were to be designated as the Mrs. Hepsa Ely Silliman Memorial Lectures. It was the belief of the testator that any orderly presentation of the facts of nature or history contributed to the end of this foundation more effectively than any attempt to emphasize the elements of doctrine or of creed; and he therefore provided that lectures on dogmatic or polemical theology should be excluded from the scope of this foundation, and that the subjects should be selected, rather, from the domains of natural science and history, giving special prominence to astronomy, chemistry, geology and anatomy.

It was further directed that each annual course should be made the basis of a volume to form part of a series constituting a memorial to Mrs. Silliman. The memorial fund came into the possession of the Corporation of Yale University in the year 1901; and the present volume constitutes the eighteenth of the series of memorial lectures.





## PREFACE

**I**N the first edition of this book I ventured the prediction that the study of capillaries and their reactions would show a vigorous growth. This prediction has been fulfilled to a degree which has even exceeded my expectations with the result that this second edition is in many respects a new book. A wealth of new information on the problems discussed has had to be incorporated, but it is of far greater importance that, mainly by the work of Thomas Lewis and his school and of E. M. Landis, new points of view have been obtained, new problems have arisen and the science of the capillaries has made a definite step forward from the descriptive stage to that of exact measurement.

I am indebted to the authors and publishers, who have kindly allowed me to use their published or unpublished figures. Professor T. R. Parsons has revised the manuscript and given me many valuable suggestions and Doctor Rehberg has helped me in every stage from the first collection of literary material, through control observations and numerous discussions of difficult points, down to the final revision of the index.

To all these I offer my sincere thanks.

The manuscript for these lectures was finished and sent to the Yale University Press on July 1, 1928, and the literature has been taken into account only up to that date.

The spelling adopted is not mine but is in accordance with the established style of the Yale University Press, which causes also on other minor points deviations from my intentions.

AUGUST KROGH

*Copenhagen,  
November, 1928.*



# CONTENTS

## PREFACE

I. INTRODUCTORY—THE FLOW OF BLOOD IN THE MICROSCOPIC VESSELS	1
The normal flow of blood	3
The pulse in the small vessels	4
The axial flow of corpusclés	5
The deformation of red corpuscles in capillaries	7
The nonexistence of "vasa serosa"	11
Irregularities of the flow of blood in the smaller vessels	12
Concentration of red corpuscles and stasis	14
Diapedesis of red corpuscles	15
Circulation and emigration of white corpuscles	16
Notes	19
II. THE DISTRIBUTION AND NUMBER OF CAPILLARIES IN SELECTED ORGANS	22
The blood vessels of muscles	25
The capillaries of the central nervous system	31
The supply of blood to the human skin	32
The capillary system in the intestinal villi	36
The capillary surfaces available in the glomeruli	40
The rete mirabile annexed to the oxygen gland in the eel	42
A plea for the study of quantitative anatomy	46
Note	46
III. THE INDEPENDENT CONTRACTILITY OF CAPILLARIES	47
The older experiments on capillary contractility	48
The modern study of capillary contractility	53

The effect of internal pressure on the caliber of capillaries	68
Note	69
IV. THE STRUCTURE OF THE CAPILLARY WALL	70
The existence of contractile cells in the capillary wall	72
The changes in the endothelium by contraction and dilatation	81
A discussion of recent studies	82
The development of capillaries	94
The specialized blood vessels of certain organs	95
The direct communications between arteries and veins and their significance	101
Notes	105
V. THE INNERVATION OF CAPILLARIES	107
Sympathetic innervation of capillaries	107
Sympathetic tonus of capillaries	113
Dilator innervation of capillaries	115
Note	122
VI. VASCULAR REFLEXES	123
"Reflex erythema" in the frog	123
Reflex erythema in man	127
Notes	140
VII. VASCULAR REFLEXES (continued)	143
Axon reflexes in the sympathetic system	143
Long path axon reflexes	144
True reflexes involving capillaries	150
Psychic vascular reactions	153
The temperature-regulating mechanism	155
Notes	158
VIII. THE REACTIONS OF CAPILLARIES TO DIRECT STIMULATION	160
Direct and indirect capillary reactions	161



# TABLE OF CONTENTS

xi

Direct reactions to mechanical stimulation	163
Direct reactions to electrical stimulation	166
Direct responses of capillaries to heat and cold	167
Reactions to hydrogen ions	168
Certain substances produce capillary contraction	174
Notes	180
IX. THE REACTIONS OF CAPILLARIES TO DIRECT STIMULATION (continued)	
A pituitary hormone	182
Substances producing capillary dilatation	195
Capillary poisons	197
Notes	208
X. INDIRECT CAPILLARY REACTIONS	
Acute reactions	209
The reaction to increased temperature	213
Slow reactions	215
Reactive hyperemia	223
A summary of indirect reactions	238
Notes	241
XI. COMPLEX CAPILLARY REACTIONS AND THEIR SIGNIFICANCE	
Paleness of death	243
Biers white spots	245
Capillary reactions in the economy of the organism	247
Capillary and arterial regulation of blood supply	248
Reactions to blood volume changes	256
The response to activity	257
The response to injury	258
Other responses	260
The vasoneurotic constitution	260
Notes	263

XII. THE EXCHANGE OF SUBSTANCES THROUGH THE CAPILLARY WALL	266
The exchange of gases in the tissues	267
The exchange of crystalloids through the capillary wall	274
The impermeability of the capillary wall to colloids	279
The exchange of water through the capillary wall	279
The colloid osmotic pressure of the blood	283
Notes	290
XIII. THE EXCHANGE OF WATER BETWEEN THE BLOOD AND THE TISSUE SPACES	293
The capillary blood pressure	293
The hydrostatic pressure of the blood in the veins	301
The venous pump	303
Tissue pressure and filtration edema	304
Differences in capillary permeability	307
The exchange of water against diffusible substances	312
XIV. CHANGES IN CAPILLARY PERMEABILITY AND THEIR MECHANISM	315
Landis' determinations of capillary permeability	318
The absolute permeability of capillaries	326
The mechanism of increased permeability	332
Restoration of normal impermeability—A permeability hormone?	336
Note	338
XV. SOME APPLICATIONS OF THE PHYSIOLOGY OF CAPILLARIES TO COMPLEX PROCESSES IN HEALTH AND DISEASE	339
The "negative" pressure in the thoracic cavity	340
The absorption of dissolved substances from the small intestine into the blood	341
The filtration of aqueous humor into the canal of Schlemm and the episcleral veins	345

## TABLE OF CONTENTS

xiii

Glomerular filtration	347
Urticaria and inflammation	350
Circulatory shock	354
The formation and absorption of edema	357
Concluding remarks	366
Notes	367

### APPENDIX ON METHODS FOR THE STUDY OF CAPILLARY ANATOMY AND PHYSIOLOGY

Observations of capillary circulation	369
Photography	372
Anatomy of capillary systems	372
Capillary contraction and dilatation	375
The histology of the capillary wall	376
Stimulation of capillaries	378
Perfusion methods	379
The rate of diffusion through tissue membranes	382
Determination of colloid osmotic pressure	383
Determination of capillary and venous pressure	385

### BIBLIOGRAPHY

### INDEX





## LECTURE I

### INTRODUCTORY—THE FLOW OF BLOOD IN THE MICROSCOPIC VESSELS

**T**HE circulatory system of man and the vertebrate animals can be considered as made up of a small number of organs or subordinate systems, which are easy to recognize anatomically, and the functions of which are on the whole quite distinct. We have a propulsive organ: the heart; a distributing organ: the system of arteries; an organ for interchange of substances between the blood and the tissues: the capillaries; an organ for collecting the blood and carrying it back to the heart: the venous system. It is evident that the organs of propulsion, distribution, and carrying back are all subservient to the functions of exchange carried out in the capillaries, and though, of course, each of the great organs is absolutely necessary for the functioning of the whole, it will be difficult to challenge the proposition that the capillaries constitute the most essential part of the whole circulatory system. It is a little strange, therefore, to find that, far from being a favorite subject for anatomical and physiological research, the capillaries have been neglected in an extraordinary degree. Though about two hundred years have passed since the capillaries were discovered you could until recently find them dealt with in a few lines in most textbooks on physiology, and the references to their structure, given in textbooks of histology, were likewise of the most summary character.

In the last twelve years, however, the capillaries have been, so to speak, "rediscovered" as a subject worthy of study and experimental research. Interest in them must have been "in the air," for the study was taken up independently and almost simultaneously in different countries and has been developed at a rapid rate by quite a number of new workers. It is now, therefore, possible and desirable to review our position, to take stock of the results obtained, to coördinate them into a sort of system, however provisional, and thereby to try and indicate fields in which further work is especially needed and to mark out lines along which progress is to be expected. This is what I shall attempt to do in this series of lectures, and I wish to say at once, in order to avoid misunderstanding, that what I propose to give is not a monograph, aiming at a complete presentation of the literature of the subject, but just lectures, in which my personal views are allowed to dominate, and the statements of the literature are dealt with in so far as they have come to my notice without any exhaustive search and in so far as I think them relevant to the problem discussed.

By way of introduction I propose to describe in some detail the observations which can be made on suitable parts of living animals brought under the microscope, but not experimentally interfered with. This should serve to give you a provisional idea of the problems to be more fully dealt with in succeeding lectures.

Observations of the capillary circulation have been made from very early times. The exquisite beauty and variety of the living pictures, presented by a number of organs when viewed in this way, have fascinated many trained observers even more deeply than they impress the casual onlooker who sees them for the first time. A large number of facts have been recorded and, while the method has its obvious limitations as a means

of causal analysis, it must be admitted that a great deal of insight into the processes of circulation can be gained by simple inspection.

The methods for arranging a number of organs for microscopic observation are given in the appendix (p. 369) and it is only necessary here to emphasize the difference in appearance between transparent organs or membranes viewed by transmitted light, and more or less opaque organs, the surface of which can be studied by reflected light. In the first case only, is it possible to study the whole of the circulation from (microscopically) large arteries to the corresponding veins and to correlate observations on capillaries s.str. with changes taking place in larger vessels. In the latter case the fragments of the circulatory system which can be clearly seen are generally too small to admit a definite interpretation.

Examining at a power of, say, 50 diameters transparent organs like the web, tongue, bladder, or thin muscles of frogs, the ears of small white mammals, the wings of bats, mesentery with small glands of various animals, one notices first a meshwork of capillaries in which the blood corpuscles are flowing at very variable rates. The capillaries coalesce into venules and veins which are usually very conspicuous. Provided the tissue has not been stimulated by any interference and is in a resting condition, the corresponding arteries and especially the arterioles are generally very narrow and sometimes difficult to find. The bore of an arteriole may be as narrow as that of a single capillary, but more often it is about double that of a normal capillary. The relatively thick walls and the extremely rapid current are responsible for the fact that they are less conspicuous than the smaller vessels.

On hydrodynamic principles the rate of flow is inversely proportional to the transverse section of the

bed, and in accordance with the observations given above the rate of flow in the capillaries is seen to be slow compared with that in the arterioles. The difference in rate between the capillaries and the venules on the other hand is not at all pronounced, though in most systems the current certainly becomes more rapid in the veins than it is in the capillaries. The general picture differs in a very significant way from that presented by an injection preparation. One is often reminded of a relatively broad stream (running at first in a number of separate channels) supplied by a system of pipes—the arterioles—and it is impossible to doubt that the main resistance to be overcome lies in the arterioles where, therefore, the main fall in pressure must take place. That this is generally so will be shown quantitatively in a later lecture (XIII) where pressure measurements will be dealt with. There are exceptions, however, to be observed for instance in the tongue or mesentery of the frog in which the arterioles are wider, the capillary bed narrower and the rate of flow from arteries to veins more uniform.

*The pulse in the small vessels.*

When a fairly large artery is watched under a low power it is sometimes possible to observe the changes in diameter corresponding to the pulse. On sinuous arteries definite movements of the loops due to the same cause are of frequent occurrence, and such movements are shown in the cinema film photographed in my laboratory to illustrate these lectures (Krogh and Rehberg, 1924). In the smaller arteries and arterioles the pulse causes no visible movement of the walls, but only variations in the velocity of the flow. Although these variations are often considerable the mean rate of flow is normally so high that the corpuscles can be seen only as stream lines during the whole cardiac cycle, and the velocity variations cannot be made out.

In the capillaries the pulse does not as a rule cause any variation in diameter of the vessels, but the velocity variations can often be very distinctly seen and are practically always present when the flow is too rapid to render them visible, as shown by Heimberger (1925)<sup>1</sup> for the human nail fold capillaries. The pulsating flow is generally equalized in the venules, because the capillary paths are of unequal length; the phases of the pulse waves arriving are different and the pulse is extinguished by interference. Occasionally, however, the capillary network is sufficiently uniform to allow the pulse to penetrate to the small veins and this may be the case especially when the pulse amplitude is exceptionally high.<sup>1</sup>

*The axial flow of corpuscles.*

When, at a fairly high magnification, the walls of the small vessels are distinctly visible it is seen that in all vessels having a diameter of two corpuscles or more the red corpuscles are flowing in a definite axial stream, surrounded by a layer of clear plasma. In somewhat larger vessels the occasional white corpuscles are on the outside of this stream and they generally show a rolling movement.

It is well known that in a fluid moving along a tube or channel, the velocity increases regularly from the resting layer along the wall toward the axis of the tube. Particles suspended in the fluid will, therefore, obtain a greater velocity on the side nearest the axis. Their movement will tend to be rolling and they will be drawn in toward the axis as can be shown in experiments on blood or artificial suspensions flowing along capillary glass tubes (Schklarewsky, 1868; Fülleborn, 1925; Fåhræus, 1928).

The extent of the zone of clear plasma depends upon the rate of flow and the size of corpuscles and vessel.



It can be stated that as soon as a tube is wider than the diameter of the red corpuscles these travel along without generally touching the wall, provided the rate of flow exceeds a certain minimum.<sup>2</sup> In larger vessels the plasma zone widens and may become nearly as broad as the largest diameter of the corpuscles.

Fülleborn (1925) has made some interesting experiments on the flow of filariform *Strongyloides* larvae in glass tubes and in pieces of arteries with a number of branches going off in different directions. These

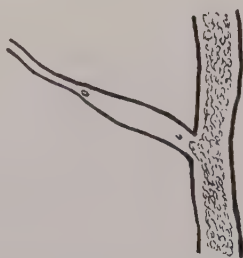


Fig. 1. Plasma skimming by contraction of a branch of an artery.

larvae have a length of  $530\mu$  with  $15\mu$  thickness. When flowing in tubes of 1.5 or 1 mm. diameter they occupied an axial stream of 0.95 and 0.45 mm., respectively, leaving a plasma zone  $275\mu$  broad in both cases. When the suspension flowed in an artery very few larvae would enter small branches going off at right angles, and the bulk would be reserved for the final branches in which the artery would split up. Similar though less pronounced variations will take place with regard to the distribution of erythrocytes in the smaller arteries and are not without significance when blood-samples are taken to determine the red cell count.<sup>3</sup>



A special reduction in the number of corpuscles, which may even amount to their complete washing away from a certain capillary field is sometimes brought about by the process which I have termed *plasma skimming* (Krogh, 1921): When a small artery branching from a larger vessel is partly contracted as indicated in the diagram, Fig. 1, the current of blood through it may seem to cease altogether, and at the same time the corpuscles are washed out from the corresponding capillaries which become empty and can easily be supposed to have contracted. In favorable circumstances it can be observed how at each pulse the column of corpuscles bulges into the mouth of the branch artery and retreats again immediately afterward, leaving only a few corpuscles which become detached and are passed swiftly along through the contracted portion of the artery. Plasma skimming is a further development of the unequal distribution of corpuscles between the smaller arterial branches, referred to above, and there is, of course, a complete gradation between a branch artery giving clear plasma and an end artery showing a cell number well above the normal.

*The deformation of red corpuscles in capillaries.*

The capillaries in a microscopic field often vary considerably in diameter. When examined under a fairly high power, so that the capillary walls can be distinctly seen, some are found which allow the corpuscles to pass in a continuous current, and these generally exhibit a definite axial stream surrounded by a plasma zone through which a white corpuscle will occasionally come rolling along. Others are so narrow that the corpuscles have to pass in single file and come continuously in contact with the wall. Others again are even narrower, and the corpuscles can pass only in a de-

formed state. The simplest deformation is observed in capillaries down to about  $4\text{--}5\mu$  diameter (in mammals) where the edges of the flat disklike corpuscles are bent in (Fig. 2, 5) while the length of the corpuscle measured during the passage does not exceed its diameter in the free state. In still narrower capillaries the red

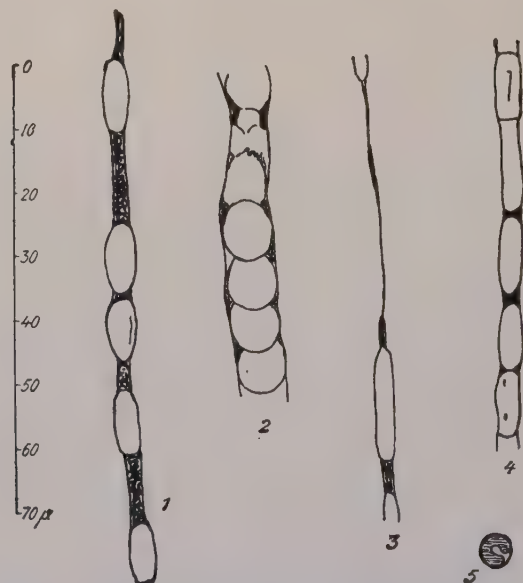


Fig. 2. Muscle capillaries from guinea pig, vitally injected with Indian ink. Walls of capillaries not shown.

corpuscles are greatly deformed and compressed into a shape like sausages the length of which may be double the normal diameter. Fig. 2 shows such corpuscles from muscle capillaries of a guinea pig, vitally injected with Indian ink. When they escape from such narrow vessels their shape immediately becomes normal again and free deformed corpuscles are never observed.

The pressure necessary to bring about the deformation in narrow capillaries must be comparatively low, since the flow does not stop in a single narrow capillary, even when the same arteriole supplies several others through which the corpuscles can pass freely, but a definite estimate cannot be attained.

It is well known that red corpuscles will pass through filter paper, the pores of which will hold back quantitatively precipitates consisting of particles which are much smaller than the corpuscles. There can be no doubt, though the passage has never been directly observed, that the corpuscles are greatly deformed during the passage. The pressure available for such a passage cannot exceed the height of fluid in the funnel.

The most direct evidence of the wonderful plasticity and elasticity of red corpuscles is obtained when they are watched in a current, where they can be caught against a projecting edge and bent by the pressure of the current flowing past them. This happens very often in the lungs where the circulation is of a peculiar character and appearance. The small arteries open through holes or very short branches into a close-meshed network of comparatively wide capillary channels on the surface of the alveoli. These channels occupy a very large percentage of the surface, but in the meshes there are small cellular islands of very varying shape. With each beat of the heart the flow in the capillaries becomes greatly accelerated, while it slows down gradually between beats.

On the cinema film of the flow of blood through the alveolar capillaries in the frog's lung, we find a corpuscle caught on a very sharply projecting edge, as shown in Fig. 3, copied from the film. It remained hanging on the edge during about four seconds (seventy pictures). At first it is riding nearly along

the short axis, and both ends are bent down in the direction of the current, but the pressure is not sufficient to press the content of the corpuscle out toward both ends. Somewhat later it slides along toward one of the ends and becomes rather sharply bent into the shape of a large and a small sac. Finally, when the current becomes slack in the interval between two beats of the heart, the corpuscle slides off the edge. The four lower figures show four consecutive stages of this

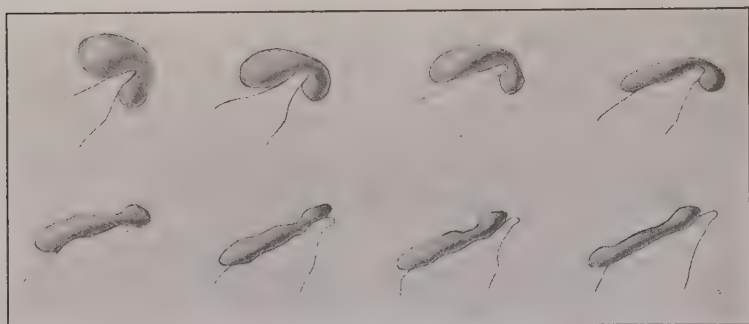


Fig. 3. Red corpuscle riding on projecting edge in frog's pulmonary capillaries and finally sliding off in four lower pictures.  
From moving picture film.

process with an interval of 0.06 second. In the last figure, about 0.3 second after the release, the shape has practically returned to normal, in consequence of the elastic properties of the corpuscle.

When a riding corpuscle is watched on the cinema film or directly through a high-power microscope, the current appears to be so rapid that it is not very surprising to see such effects, but it must be remembered that the rate of flow is magnified in the same proportion as the objects. On the film the actual rate can be approximately measured by noting the shifting posi-

tion of free swimming corpuscles from one picture to the next. In the case now described, the rate, during the period the corpuscle remained hanging, did not exceed 0.12 mm. per second, corresponding to about 400 mm. per hour, a rate which is too slow to be followed by the naked eye. I am not familiar enough with hydraulic problems to venture upon a calculation of the actual pressure to which a current of this rate has exposed the corpuscle, but it is evident that it must be of a very low order.

*The nonexistence of "vasa serosa."*

Capillaries may sometimes be so narrow that they cannot admit red corpuscles even when these are compressed ad maximum. Such "empty" capillaries were observed early in the last century and have played a prominent part in the discussion on the peripheral circulation under the designation "*vasa serosa*." Even as late as 1888 Cohnstein and Zuntz described *vasa serosa* as normal constituents of the frog's web, and in recent papers (Smith, Arnold, and Whipple, 1921) their possible existence is sometimes used as an argument to explain discrepancies in blood counts, etc. It should be pointed out, however, that capillaries can only under exceptional circumstances and for short periods admit a slow current of plasma without admitting corpuscles. This is an unavoidable consequence of the extreme softness of the red corpuscles. When a capillary containing one or more red corpuscles contracts to such a degree that the corpuscles cannot pass along, they will be squeezed into a form which fills up entirely the lumen of the vessel and prevents any passage of plasma along it. It is only when a capillary happens to contain no corpuscles at the moment of becoming so narrow that these elements can no longer be squeezed through, that it may for a time admit a current of



plasma which must necessarily be very slow. Even in that case it will generally not last long before a corpuscle is carried into the mouth of the vessel and blocks the passage. In my observations of the circulation in specimens vitally injected with Indian ink, I have found that the submicroscopical particles of this substance can pass, as a rule, through only those capillaries which are also open to the passage of corpuscles.

*Irregularities of the flow of blood in the smaller vessels.*

When a microscopic field in any organ is watched for some time many slight variations in the flow are usually observed. These are due to local contractions and dilatations, very often too slight to be directly observed or measured. In single capillaries the flow may become retarded or accelerated from no visible cause; in capillary anastomoses the direction of flow may change from time to time. Similar changes in the direction of flow are sometimes seen also in arterial anastomoses and more often in veins. The mechanism of all these apparently spontaneous changes is unknown, but their effect certainly is an extremely nice adjustment of the general uniformity of the circulation. When an organ like the tongue of a frog is pinned out the mechanical tension on the different parts varies greatly, and one would expect certain areas to be cut off from the blood supply, but such a thing has never happened within my experience, and whether a membrane is stretched, folded, or bent in sharp angles the blood finds its way to any part where the actual pressure in the tissue does not exceed the pressure available in the arteries supplying that part.

Cases have been described, especially with regard to the nail capillaries in certain patients (Hinzelmann, Otf. Müller, 1922), in which the flow at frequent, but



quite irregular, intervals, often several times per minute, will stop altogether to resume its course after a period of one to many seconds. Sometimes the flow will come to a simultaneous standstill in all the capillaries in one nail, sometimes the flow stops only in a small group of capillaries. This is a further and pathological development of what is seen normally in most tissues as well as along the nails of human subjects, viz., irregular variations in the tone of arteries of different size. The condition obtaining when an artery has contracted completely is called stasis by several authors, but this terminology is very unfortunate, leading as it does to confusion of a simple suspension of flow with the quite different condition of true stasis, characterized by the packing of corpuscles presently to be described.

When the flow in the capillaries and small veins is slowed sufficiently it is often, in observations on mammals and especially on man, described as granular, and when the flow stops the corpuscles are seen to agglutinate into large lumps with spaces of clear plasma between them. These phenomena constitute an *in vivo* demonstration of the agglutination taking place in shed blood and brought back from oblivion by the beautiful researches of Fåhræus (1921) on the "Senkungsgeschwindigkeit" of red corpuscles. With a rapid flow the turbulence is sufficient to counteract the mutual attraction and adhesiveness of the corpuscles, which bring about agglutination when the blood is quiescent.

Even when agglutination is very complete the apparently massive columns of corpuscles offer very little resistance and are swept away into the veins and broken up as soon as the flow is resumed (Tannenberg, III, 1925). True stasis, which shows a striking similarity to the complete agglutination described in so far

as the red corpuscles are seen to be closely packed, shows a very different form and degree of resistance to the resumption of flow.

*Concentration of red corpuscles and stasis.*<sup>4</sup>

At a fairly high magnification it is sometimes possible to observe how the corpuscles become concentrated during the passage of a long capillary. Where they enter, the single corpuscles can be seen to be a certain distance apart, and there is a distinct zone of clear plasma along the wall. During their progress they come much closer to each other and to the walls. This is the first stage leading up to the definitely pathological condition of stasis.

The development of stasis can be most easily observed in the tongue and mesentery of the frog and in the mesentery of mammals (Krogh and Harrop, 1921; Florey, 1926, 2; Tannenberg, III, 1925). In the first stage the corpuscles at the venous end of a capillary become so closely packed that they obstruct the flow, and during the further development we see the blood enter into this capillary in a distinctly pulsating flow; the fluid part of the blood disappears during its progress, and the corpuscles are deposited on the already existing column which grows until the whole of the capillary is filled up. The process is well shown as a result of urethane application to the tongue of a frog in the cinema film.

The column of corpuscles is generally transparent, which shows that the corpuscles are so closely packed that the rays of light can pass through without suffering refraction at the surfaces of the single corpuscles. In hematocrite tubes this transparency is taken as an indication of complete separation of the corpuscles from the plasma, and no amount of centrifuging will reduce the length of a column of corpuscles so packed.

The corpuscle volume determined in this way corresponds closely to that found by indirect methods, and it must be concluded, therefore, that in a transparent column plasma is practically absent, in spite of the doubt expressed by Tannenberg (III, 1925), who believes that agglutination of corpuscles is the main factor responsible for stasis instead of loss of plasma.

Stasis appears often, or perhaps usually, to be an irreversible process, but Florey (1926, 2) has had the opportunity of observing in the rat's mesentery the resolution of stasis in a number of capillaries. A main factor appears to be the capillary pulsation which will cause a gradual loosening of the massed corpuscles at the arterial end of the column, which loses its transparency, while at the same time the corpuscles are broken loose one by one or in small lumps by the pulsating fluid. In other cases the whole length of a column becomes imbibed with fluid, and apparently a series of contractions gradually forces the content over the lip of the stased capillary into the current of blood, when the liberated corpuscles are washed away.

#### *Diapedesis of red corpuscles.*

When the circulation is observed in inflamed tissue, where the blood flow is slow and the capillaries generally dilated, the process of diapedesis of single red corpuscles or parts of corpuscles can be observed along with the emigration of leucocytes of which I shall presently have to speak. Both these processes were very carefully studied long ago by Cohnheim, and I refer especially to the descriptions given in his lectures on general pathology (1877, pp. 120-125, 197-200).

The red corpuscles pass out through the capillary wall sometimes quite smoothly as through a hole, and it happens that two or more corpuscles pass through

in the same place one after another. Often, however, a corpuscle may stick in the wall for some time, with the result that one part is carried off by the current of blood, while the other is finally deposited outside the vessel. In capillaries which have gone into stasis the wall of the vessel may slowly bulge out in one or (generally) more places, and suddenly it is found that the corpuscles contained in such a varicose dilatation are outside the vessel.<sup>5</sup> The most interesting point is that the integrity of the wall seems in all cases to be restored very rapidly. Even where a few corpuscles pass out one after another they are not followed by a further number, though diapedesis may take place just afterward close to the point, and when the circulation becomes normal again in a capillary which during stasis has lost a number of corpuscles in many places, the vessel appears to be tight not only to corpuscles but to the plasma fluid as well. Drinker, Drinker, and Lund (1922) have described how in the bone marrow the reverse process takes place and the newly formed red blood cells are pushed into the lumen of capillaries, the walls of which are closed behind them.

*Circulation and emigration of white corpuscles.*

The white corpuscles circulating in the blood are always spherical. Being comparatively few in number they are usually difficult to observe in the rapid stream in the arteries. They are occasionally seen to pass through capillaries, where the resistance offered to their passage through vessels with a diameter less than their own appears to be greater than that experienced by erythrocytes.

The leucocytes are most easily and most often seen in the small veins, where there is a definite axial flow and a not too rapid current. Here they are found chiefly in the marginal layer of plasma, rolling along

the wall, sometimes in a more or less straight course, sometimes in spirals, but without entering the axial stream of more rapidly flowing erythrocytes. Schklarewsky has shown by his experiments in glass tubes that the expulsion of the white corpuscles from the axial stream is a consequence of the fact that their specific gravity is intermediate between that of the erythrocytes and that of the plasma. Their movement is a rolling one by reason of the increase in velocity from the periphery to the axis of the vessels. It appears, however, as if some slight adhesion between the endothelium and the surface of the white corpuscles had also something to do with the peculiarity of the movement. At least the corpuscles are often seen to move much more slowly than the plasma and to stop completely for short periods in contact with the wall. When the tissue is in a state of inflammation the white corpuscles become definitely adhesive to the vessel walls especially in the venules, but also, though generally at a somewhat later stage, in the capillaries. They are then flattened out by the current into a pyramidal or wedge-like shape with the top against the current as described by Tannenberg (IV, 1925). At first a slight impulse like the collision with another corpuscle will be sufficient to loosen the hold and cause the adhering corpuscle to roll on, but a little later it adheres more firmly, and emigration may take place. The tip of the corpuscle bends and penetrates the wall, and slowly the whole of the corpuscle moves through the endothelium, while pseudopodia may be stretched out to a considerable distance outside.

In the circumstances which cause emigration the leucocytes are strongly attracted toward and even beyond the vessel walls, and the smaller veins may be covered inside by a layer of corpuscles several cells deep, while many capillaries become more or less com-



pletely blocked. It appears that almost all leucocytes carried to an inflamed spot are held back by the attracting forces and eventually find their way into the tissue spaces.<sup>6</sup> As in the case of the passive diapedesis of the red corpuscles the active penetration of the white leaves the endothelium substantially intact, that is, the opening, which must undoubtedly exist during the penetration, closes again immediately afterward by the elasticity or vital contraction of the cell or cells concerned.

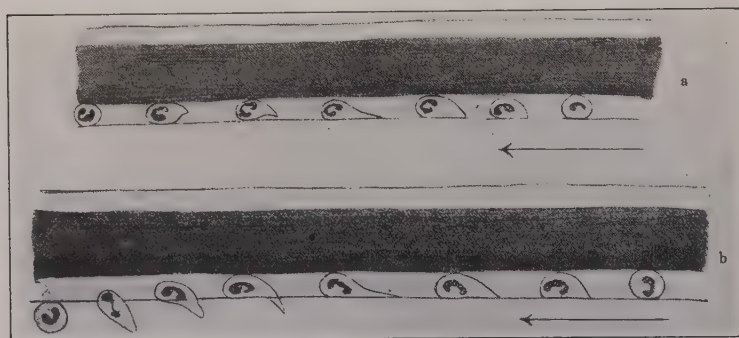


Fig. 4. Stages in the diapedesis of leucocytes. Semidiagrammatic.  
After Tannenberg.

In the present lecture I have tried to give a simple objective description of some of the outstanding phenomena of the circulation in the small vessels as they are to be observed through the microscope. As many of these phenomena will come up again for study in subsequent lectures I have endeavored here to avoid as far as possible any causal analysis.

A few points on the border line of my subject, but of sufficient interest to be treated a little more closely are referred to in the appended notes.



## NOTES

<sup>1</sup>*Clinical capillary pulsation* has been very carefully studied by Lewis (1924, Monograph, 1927, p. 267) who shows that it is not, as formerly supposed, a special symptom of aortic regurgitation, but is of regular occurrence in the skin and lips of normal persons under certain conditions. Capillary pulsation in the clinical sense is brought about when the pulse wave penetrates through the capillaries to the venules in sufficient force to cause them to dilate. Usually the pressure is not sufficient to cause "spontaneous" pulsation, but the changes in pressure become evident when the skin is lightly pressed with a glass plate so as just to make it pale during diastole. Lewis has shown that this pulsation is brought about when the arterioles become dilated, e.g., by the effect of heat, while the state of the larger vessels is generally without perceptible influence. A large difference between systolic and diastolic pressure, as in cases of aortic regurgitation, will of course favor the appearance of capillary pulsation.

<sup>2</sup>*Apparent variations in caliber of human skin capillaries.* When the capillaries of the human skin, especially at the nail fold, are viewed by reflected light the walls of the vessels remain absolutely invisible, and only the stream of corpuscles can be seen. Although competent observers (Heimberger, 1925) have warned very often against drawing conclusions regarding the vessels from the changes in width of the moving columns of corpuscles, this error is made again and again. Descriptions have been given for instance of peristaltic waves traveling along capillaries which were nothing but occasional breaks in the stream of red corpuscles.

In a recent paper Crawford (1926) employing a beautiful cinematographic technique has described irregular local changes in diameter of the nail fold capillaries taking place with such velocity that marked differences can be measured between one picture and the next (in 1/15-1/20 second). As with ocular inspection only the moving column of corpuscles can be photographed and measured on the cinema film, and what has been studied are the irregularities occurring in this column.

<sup>3</sup>*The red cell count in "capillary blood."* Fåhræus (1928) has pointed out and verified experimentally the interesting consequence of the larger axial velocity, that blood flowing along a capillary tube will be more dilute with respect to corpuscles than that in the larger vessels. The difference is pronounced in all vessels below  $\frac{1}{2}$  mm. diameter down to capillaries of a diameter equal to or less than that of the corpuscles. If blood is flowing from one reservoir to another through a capillary tube the composition in both reservoirs will, of course, be the same, but the blood present at any moment in the capillary will be more dilute, and Fåhræus found a reduction in the red cell count from 5 to 4 million in a tube of 0.1 mm. bore and to 3.5 million, when the bore was reduced to 0.05 mm.

It is very important to note that it is only the blood flowing in the small vessels which is different from that in the larger arteries and veins, while a sample flowing out, say from an incision, becomes normal

again. The discrepancy may affect the determination of total blood volume as assumed by Smith, Arnold, and Whipple (1921), and also by Fåhræus, though in my opinion the effect is probably too small to be appreciable, but in principle it does not influence the composition of samples of so-called capillary blood taken by skin incisions.

Nevertheless, a certain amount of caution is necessary in taking such samples.

When a sudden incision is made with a sharp instrument in the lobe of an ear the blood will flow out mainly from the arteries which have been cut, while the capillaries and venules will contribute only an insignificant amount, because the pressure in capillaries and veins at levels above the heart is normally extremely low. At the moment of section a sudden fall of pressure is produced in the arteries concerned, and the drop of blood shed will represent mainly the axial stream rich in corpuscles. It has been observed again and again that sampling from this first drop is apt to give too high counts for the red cells, and it is a general rule, at least in Denmark, that this drop shall always be discarded.

A few seconds after the incision the divided vessels begin to react and there is reason to believe that their normal reaction is a contraction, proceeding from the wound toward the larger vessel from which the cut branch is given off. This contraction will often produce plasma skimming; the resulting counts are then likely to be too low, and this is especially the case when the flow diminishes rapidly. Consistent and correct results can be obtained when an active hyperemia is produced in the ear and the incision is made with a very sharp instrument giving a minimum of stimulus toward contraction of the arteries. The blood should flow freely, and it is much better to take samples of 0.1 cc. and mix in a small test tube, according to the method of Ellerman and Erlandsen (1910), than to use the special Thoma-Zeiss mixing pipettes on samples of a few c.mm.

In a paper by Duke and Stofer (1922) figures are published which appear to show that in pernicious anemia the "capillary" blood becomes highly concentrated. Comparing counts from the lobe of the ear with simultaneous counts from an arm vein they found an excess of 0.3 to 1.3 million red cells (average 0.8 mill.) per c.mm. in the "capillary" blood from their patients, while in normal subjects or in secondary anemias no significant difference could be observed. The authors ascribed the discrepancy to sedimentation of corpuscles in the small vessels, but this to me seemed out of the question, and I found it necessary to have their observations repeated. Dr. E. Meulengracht, chief physician to the Bispebjerg Hospital in Copenhagen kindly undertook to test a small number of patients suffering from pernicious anemia by making a series of almost simultaneous counts and hemoglobin determinations from right and left ears, fingers, and cubital veins. The results show no significant difference in any case, and the general averages are for the ears 48 per cent hgb. and 1.80 mill., fingers 47 per cent hgb. and 1.80 mill., veins 46 per cent hgb. and 1.79 mill. red cells. Thanks to Dr.

Meulengracht it can be definitely stated that when samples are taken and treated with proper care reliable and consistent counts and hemoglobin estimations can be obtained from capillary blood in pernicious anemia as well as in other cases.

<sup>4</sup> Etymologically the word stasis refers to a flow which is brought to a standstill not by a lack of driving force but by an impediment met with. Stasis has been known for a long time as occurring in inflammation and has been shown to be due to a packing in the small vessels of either red or white corpuscles. In this sense the word has been used since Cohnheim (1867), while its use to denote a simple suspension of flow seems to be quite recent.

<sup>5</sup> *The fate of extruded erythrocytes.* Reference should be made here to the beautiful observations of E. R. and E. L. Clark (1909, 1926). These authors have produced small bleedings in the transparent tail of young tadpoles. They have seen how lymphatic vessels in the immediate vicinity of such extruded corpuscles send out sprouts which grow toward the corpuscles and absorb them into their lumen, whence they are carried by the lymph flow back to the blood. This process begins a few hours after an extravasation. At the largest distance at which the reaction was observed (76 microns) the lymphatic sprouts took 48 hours to reach the corpuscles, which were then taken in during 5 hours.

During the first 15-24 hours after extrusion the erythrocytes are immune to the attacks of pigmented wandering cells, but after that period they are taken up and digested, and this fate befalls all erythrocytes, which are too far removed from any lymphatic vessel to stimulate it to sprouting. In very young larvae (up to one week) the blood capillaries themselves are apparently stimulated by the presence outside their walls of red cells and may send out sprouts to absorb them, but this power is lost in somewhat older animals.

<sup>6</sup> *Emigration of white corpuscles.* Many attempts have been made to explain the diapedesis of white corpuscles as the result of relatively simple physical or physico-chemical causes, such as changes in blood pressure, plasma viscosity, specific gravity, and surface tension of the leucocytes. An instructive discussion of these possibilities has been given by Tannenbergl (IV, 1925). Apart from the fact that the reality of the changes postulated is largely hypothetical, it should be pointed out that they fail utterly to recognize the central problem in the process, viz., the "chemotactic" attraction of leucocytes by certain substances and notably by products of bacterial metabolism. When in a microscopic field, containing in vitro blood with living leucocytes and bacteria, we see a leucocyte at a certain distance from a bacterium become suddenly active and move directly toward the bacterium, pushing the red cells in its way to the right and to the left, we observe the process which is mainly responsible for emigration of leucocytes from the vessels. Not until this problem has been solved by reduction of the process to well-known chemical and physical causes is it worth while to speculate on the still more complicated mechanism of emigration from the blood current through the walls of the vessels.

## LECTURE II

### THE DISTRIBUTION AND NUMBER OF CAPILLARIES IN SELECTED ORGANS

I GAVE in the first lecture a very general description of the capillary circulation as seen under the microscope, and I want now to introduce to you the numerous and complicated problems involved in an analysis of these simple observations. This can be done in several different ways. The problems of physiology are intimately connected with each other and with those of anatomy and pathology, but there is no definite order in which they have to be presented in order to give a logical and coherent picture. The order is always a matter of personal choice, and whatever choice is made it will turn out to have its serious drawbacks and involve the anticipation of explanations to be given later on and the separation of subjects which are too intimately connected in nature to be with impunity separated in a description. Repetition will be necessary to remedy some of the more obvious defects in the line of argument.

These lectures being, as I have said, of a personal character I think my best course of action will be to proceed mainly along the line which I have followed myself and begin by stating briefly the problem with which I was confronted twelve years ago, when I began seriously to study the physiology of capillaries. My problem was the supply of oxygen to the fibers of striped muscle, the mechanism by which it was



brought about and, especially, how it could be regulated.

The oxygen is presented to muscle fibers in the blood running through the capillaries which traverse the tissue. By whatever means the transport of oxygen from the capillaries to the muscle elements is brought about, it is clear that the facility of transport must be related to the number and distribution of the capillaries and related, further, to the permeability for oxygen of the capillary walls and the tissues themselves. An essential part of my task must, therefore, be to try and obtain information on these points.

Muscles do not use oxygen at a constant rate. What Barcroft in his admirable book, *The Respiratory Function of the Blood* (1914), has aptly termed their "call for oxygen" is variable in the extreme. During heavy work they may take up ten to twenty times more oxygen than during rest, and certain observations might even suggest that their power of doing work was limited by the supply of oxygen which they were able to secure for themselves. However that might be, it seemed to me clear that there must be some mechanism regulating the conditions of supply. With constant conditions the facilities for transport must either be ridiculously out of proportion to the requirements of the muscles during rest or ridiculously inadequate to meet their needs during heavy work.

You will perceive at once, and I need not emphasize it by superfluous words, that the problem, of which I have just sketched the outlines, constitutes a more or less representative part of a more general problem: By what forces and by means of which mechanisms is the function of capillaries, viz., the exchange of substances between the blood and the tissues or tissue-fluids, carried out, and how is this function regulated

by the organism, adapted to its ever changing needs and coördinated with its multifarious activities?

In order to solve this general problem or the more modest part of it, which had forced itself upon my mind in 1915, information of a very varied nature must be brought together; but, as I said just now, the first thing to do must be to obtain an idea of the number, distribution, and surface of capillaries in those tissues in which we are interested.

For information of this kind we naturally turn to the anatomical literature, but I regret to have to say that in the main we are disappointed. We cannot find there that quantitative information which we require. We will find a certain number of papers in which the distribution of capillaries in different organs is described, and we will find numerous figures illustrating the distribution, but the capillaries had practically never been counted, and the illustrations, from which at least approximate countings could in many cases be made, are as a rule deficient in that one respect which is for our purpose the most essential: the magnification is not given at all, or is given in such an ambiguous way that it is impossible to be sure whether it is the actual magnification of the figure as published, the magnification of the original drawing (which is usually arbitrarily reduced in reproduction) or merely the magnification of the microscope employed, which is meant. It is a great pity that so many beautiful and excellent anatomical and histological drawings, which have often cost a tremendous amount of the most painstaking labor, should be next to useless for physiological purposes, just because the means are wanting by which to ascertain the actual size of the structures or elements depicted, and it is the greater pity, because the want could have been so easily supplied, had it only been realized. We are all aware that the efficiency of an



engineering structure depends not only on its form but also on its actual size, that the structures by which a ditch is successfully bridged are absolutely inadequate for a broad river, and that a uniform increase in the linear dimensions of a house and its furniture of, say, 20 per cent would make it extremely uncomfortable for human beings to live in; but very few people seem to realize that the same holds also for the microscopic structures of the organism, which could not function at all if magnified to the size given in our pictures, while the function of a large number of them would be very greatly impaired by even a slight deviation from their actual size. I shall have more to say later on this important point, and I hasten now to add that it is not, of course, my intention to belittle the work done by anatomists, but only to emphasize the quantitative point of view and to offer an explanation of why it is not at present possible to give more than a few rather crude examples of the application of quantitative principles. It is only right to add, further, that in most tissues the arrangement of capillaries is so complicated that the difficulties in the way of even approximate measurement are very formidable.

I have brought together, partly from the literature, partly from investigations undertaken in my laboratory, some quantitative information—all of it preliminary and imperfect—about a few capillary systems which are of importance physiologically and to the functions of which I shall have to return later in the course of these lectures, and I shall begin by describing the capillary system of striped muscles, which is comparatively simple and comparatively well known.

#### *The blood vessels of muscles.*

The arrangement of the blood vessels in striated muscles has been studied and depicted very carefully

by Spalteholz (1888). The arteries supplying a muscle branch freely, and between the branches there are very numerous anastomoses forming a primary network. Into the meshes of this net small arteries are given off at regular intervals, and these again anastomose freely, forming a secondary cubical net of great regularity. From the threads of this network the arterioles branch off, generally at right angles to the muscle fibers and at very regular intervals (of about 1 mm. in the warm-blooded animal), and these arterioles finally split up into a large number of capillaries running along the muscle fibers and in the main parallel to them, but with numerous anastomoses, forming long narrow meshes about the fibers. The capillaries unite into venules in-

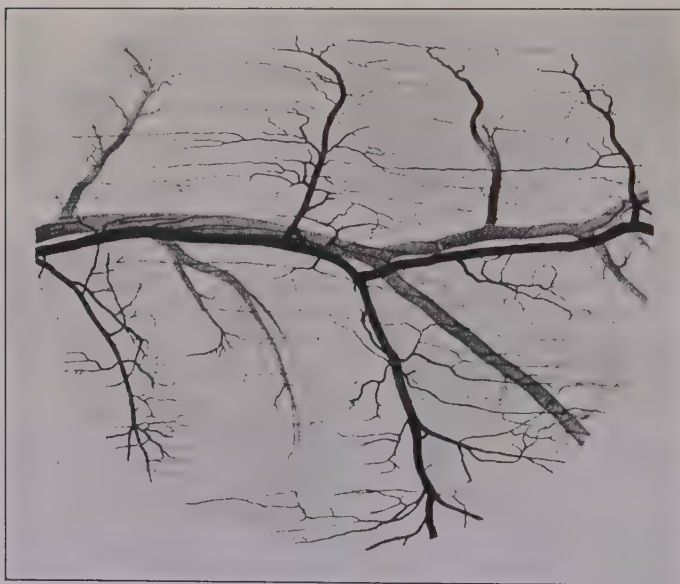


Fig. 5. Small arteries (black), capillaries, and veins from striated muscle. After Spalteholz.

tercalated regularly between the arterioles, and the whole system of veins reproduces and follows almost exactly that of the arteries. All the veins down to the smallest branches are provided with valves allowing the blood to flow in the direction of the heart only. Short pieces of secondary arteries and veins, with arterioles, venules, and capillaries, are shown in Fig. 5, reproduced from Spalteholz.

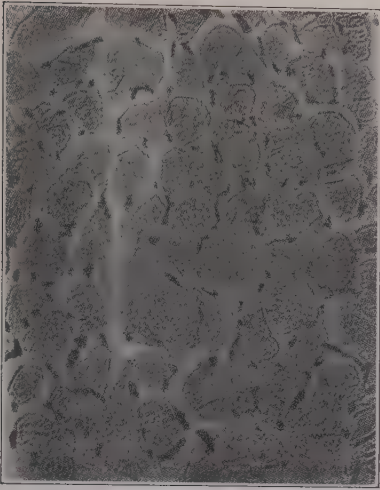


Fig. 6. Transverse section from the injected m. gastrocnemius of a horse.  $\times 156$ .

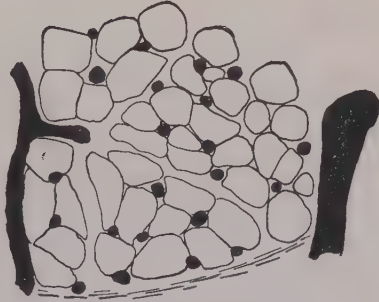


Fig. 7. Transverse section of injected muscle from the tongue of a cat.  $\times 268$ .

When the muscle contracts its form is greatly altered, the fibers becoming much shorter and proportionally thicker. The whole of the vascular system is beautifully adapted to these changes: the arterial and venous networks insure the supply and drainage of almost every point, even if a number of anastomoses are temporarily blocked. The capillaries, which in the

resting muscle are practically straight, become much twisted. The blood is driven out by compression from a number of the venous branches, and, when the muscle relaxes again, these can be filled from their peripheral ends only. Since muscular contractions usually more or less regularly alternate with relaxations, the system of valves makes of the veins of every muscle a very effective pump, capable of maintaining a low pressure in the muscle capillaries. The significance of this arrangement will come up for study later, but at present our attention must be focused on the capillaries. It is apparent from Fig. 5 that sections, cut at right angles to the muscle fibers, must represent the capillaries as dots which can be counted and the distribution of which can be studied. Such transverse sections are given in Figs. 6 and 7; and an inspection of them shows that the capillaries are present in very large numbers and are distributed among the muscle fibers with conspicuous regularity. A quantitative test of the regularity of the arrangement can be obtained by counting the number of capillaries in a large number of small equal areas chosen at random from sections of the same muscle. I give as an example a series of countings, each made on an area of 0.0300 square mm. (mm.<sup>2</sup>), from five different transverse sections of the m. gastrocnemius of a horse.

	1	2	3	4	5
	45	34	38	38	31
	40	34	42	43	33
	42	40	43	47	43
	41	46	41	49	39
	44	44	46	33	36
	36	41	..	..	..
	49	38	..	..	..
	—	—	—	—	—
Average,	42	39	42	42	36

An inspection of this table shows the remarkable regularity of distribution, and, when it is treated

mathematically, the average number of capillaries in the area measured works out as  $40.5 \pm 5$ , a dispersion of not more than 12 per cent. Dividing by 0.03 we get the number of capillaries per square mm. transverse section as not less than 1,350 with a mean error of  $\pm 31$ . The transverse section of an ordinary pin is about 0.5 mm.<sup>2</sup> It requires some mental effort to conceive how there can be room within such a pin for about seven hundred parallel tubes carrying blood, in addition to about two hundred muscle fibers.

In other animals the number of capillaries per mm.<sup>2</sup> may be even larger. It is well known that mammals have a higher metabolism than cold-blooded vertebrates, and small mammals a higher metabolism than large ones, and there appears to be some relation between the metabolic activity and the number of capillaries per mm.<sup>2</sup> of muscle. In a dog's m. semimembranosus the number worked out from 30 countings as  $2,630 \pm 51$ , while the dispersion or "standard deviation" of the single counting was not more than 10.6 per cent.

Even larger figures were found for guinea pigs' muscles, and I have no doubt that in the smallest mammals the number of capillaries per sq. mm. can be above 4,000. In cold-blooded animals, such as the cod and the frog, much smaller figures are found, averaging only about 400.

Stoel has compared (1925) the number of capillaries in white and red muscles in the domesticated rabbit. The figures given by him are remarkably low. He finds for the red m. semitendinosus only 790 capillaries per mm.<sup>2</sup> and for the white m. add. magnus 1,550, while in the heart he has counted 3,230.<sup>1</sup> The diameters given as averages are very narrow, viz., 5.2, 2.5, and 5.0 $\mu$ , respectively, but I cannot feel convinced that the diameters measured on injected and fixed specimens are



trustworthy. Quite recently (1927) Duyff and Bouman have repeated Stoel's countings and extended them to a number of other muscles in the rabbit. They report higher figures, from 1,000-2,700 capillaries per mm.<sup>2</sup> (not cm.<sup>2</sup> as they say), but give their numbers as relative only.

To obtain some insight into the meaning of such figures as those found by my countings let us consider very briefly the problem concerning the supply of oxygen to the muscular tissue. The oxygen molecules have to travel outward from the capillaries, and the longest distance a molecule has to go must be half the distance between neighboring capillaries, denoted  $R$ . This works out in the case of the frog's muscle (400 capillaries per mm.<sup>2</sup>) as  $R = 28\mu$  (calculated from the center of each capillary) and in the case of the dog's muscle (2,600) as  $R = 11\mu$ . I shall show later in some detail how these figures can be utilized for a calculation of the oxygen pressure head necessary for supplying the muscles. If we consider the exchange of dissolved substances between the blood and the muscle lymph, this depends evidently on the available capillary surface, and, assuming the average diameter of capillaries  $2r$  as equal to the diameter of a red blood corpuscle, we obtain the following figures for the total surface of capillaries in 1 cubic centimeter of muscle:

Muscle	Weight of animal kilos	Muscle capillaries per sq. mm.	$R$ $\mu$	$2r$ $\mu$	Capillary surface cm. <sup>2</sup> per cm. <sup>3</sup>	Volume per cent	Surface of 1 cc. blood cm. <sup>2</sup>
Frog,	0.05	400	28	15	190	7.1	2,700
Horse,	500	1,400	15	5.5	240	3.3	7,300
Dog,	5	2,600	11	7.2	590	10.6	5,600

On the same assumptions the volume of blood in the muscle capillaries works out as between 3.3 per cent (horse) and 10.6 per cent (dog) of the muscle volume,



and the surface of 1 cc. blood contained in capillaries as 2,700 cm.<sup>2</sup> (frog) to 7,300 cm.<sup>2</sup> (horse). It is evident that very large exchanges of substances can take place in a short time through such enormous surfaces. Supposing a man's muscles to weigh 50 kg. and his capillaries to number 2,000 per sq. mm., the total length of all these tubes put together must be something like 100,000 kilometers or  $2\frac{1}{2}$  times round the globe and their total surface 6,300 sq. meters.

It is evident that much more work could be done, and ought to be done, on what I should like to term the *quantitative anatomy* of muscle capillaries. A number of different animals ought to be examined and a number of different muscles from each animal. The regularity or otherwise of the capillary arrangement ought to be made out and definite relationships established between the capillary supply and the amount of work required of muscles. I would suggest, for instance, comparisons between the capillaries in the muscles of the hind legs and the hearts in domesticated rabbits and hares.

*The capillaries of the central nervous system.*

In a valuable series of papers E. H. Craigie (1921, 1924, 1925) has studied quantitatively the vascularity of a large number of regions in the central nervous system of the rat and made very suggestive comparisons showing the development of vascularity after birth and correlating this with the development of function of the different parts. The method, which appears to be generally applicable to tissues in which the network of capillaries is irregular and complex, will be discussed in the appendix (p. 374). The results are here corrected and given as the total length of capillaries in mm. in a cube of 1 mm.<sup>3</sup>, a figure which can be directly compared with the number of capil-

laries per sq. mm. transverse section of striated muscle. It is shown that at birth there is little difference between the regions and indeed between white and gray matter, all showing about 400 mm. of capillary per cubic mm. of tissue. During growth a differentiation sets in, and the differences in vascularity between the different centers, which are characteristic of the adult, become established between the tenth and twenty-first days. Later there is a decrease in vascularity in several of the centers of the brain stem, while in the cerebral cortex the capillary supply continues to increase. The average figures for the cortex are at birth 430, at the age of 10 days 530, 21 days 1,200, 90 days 1,400, and 390 days 1,470 mm. per mm.<sup>3</sup> The rapid development coincides with the period of weaning when the sensory and motor activity of the brain progresses also at a rapid rate. The figures for the adult cortex while falling short of those for muscle are remarkably high and point to the existence of a high rate of metabolism.

*The supply of blood to the human skin.*

The vascular system of the skin has been very carefully studied by Spalteholz (1893, 1927) and illustrated by numerous figures, the magnifications of which are correctly given, as Professor Spalteholz himself has very kindly informed me.

The skin is supplied from the underlying tissue through a large number of small arteries. In all places where the skin is movable these arteries are very sinuous and will be able to supply blood even when greatly stretched. In the deepest layer of the cutis the arteries form a richly anastomosing irregular plexus, from which small arteries rise perpendicularly through the skin, to form, a little below the papillae, the *sub-papillary arterial plexus*. This plexus is on the whole

regular, with oblong meshes, more or less parallel to the papillary ridges. The meshes are of somewhat different size in different regions, varying from 0.2 to 2 mm.<sup>2</sup> They are smallest in the hand and foot, where the skin is regularly exposed to pressure.

From the subpapillary plexus still smaller arteries spring to supply the papillary capillaries. These do not anastomose, and each supplies a small but variable

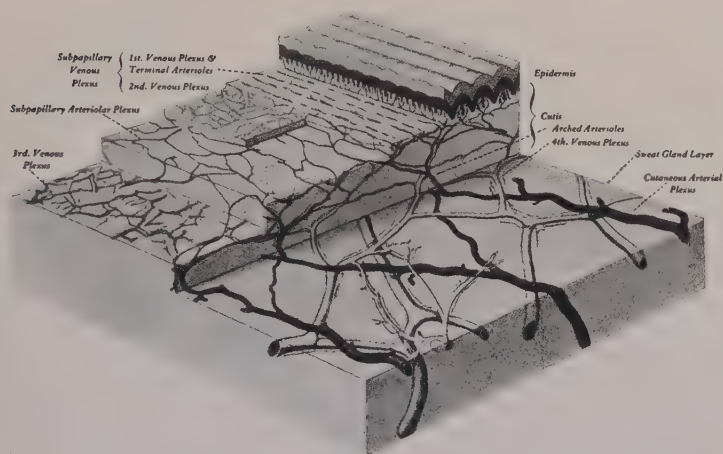


Fig. 8. Diagram of the skin and its vessels. After Spalteholz.

number of papillae. Spalteholz has found the area supplied to vary in the sole of the foot between 0.04 and 0.27 mm.<sup>2</sup>

Each papilla is normally provided with a central capillary loop, the arterial branch of which is generally narrow, while the tip of the loop and the venous branch are often 0.02 mm. or more in diameter. The length of the loops varies generally from 0.2 to 0.4 mm.

That the number of capillaries is very small com-

pared with that of the muscles is shown by the investigations of Wetzel and Zotterman (1926) who have made numerous countings of the capillary loops in different regions of the human skin and give as averages per mm.<sup>2</sup> for the dorsum of the hand 64, forearm 47, cheek 16, and circumoral skin 20. In the cheek the small number is compensated by a large size as shown by a comparison between Figs. 9 and 10, but in the circumoral skin the capillaries, though few, are exceptionally narrow.

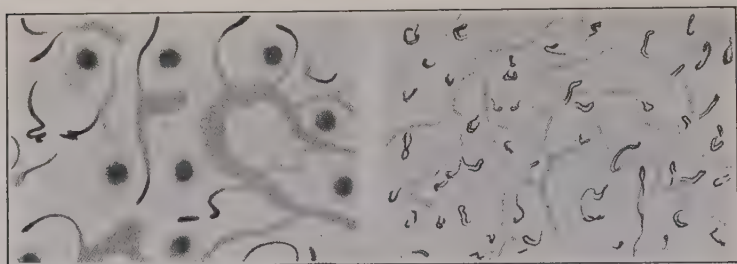


Fig. 9. Skin vessels from forearm.  
After Wetzel and Zotterman.

Fig. 10. Skin vessels from cheek.  
After Wetzel and Zotterman.

The venous branches of the papillary capillaries combine to form venules which return to a first *subpapillary plexus* of small veins, situated just below the papillae. This plexus is partly shown in Figs. 9 and 10. The venules by which it is made up are of about the same size in each region and usually only a few hundredths of a mm. wide.

The first subpapillary plexus of veins is connected by very numerous short anastomoses with a second close-meshed network, lying at about the level of the arterial subpapillary plexus and made up, like the first, of very narrow venules.

Passing down through the cutis Spalteholz describes

two more plexuses with larger meshes made up on the whole of larger veins. In the last of these, lying in the boundary zone between the cutis and the subcutis, valves begin to appear, while in all the veins throughout the skin itself valves are absent.

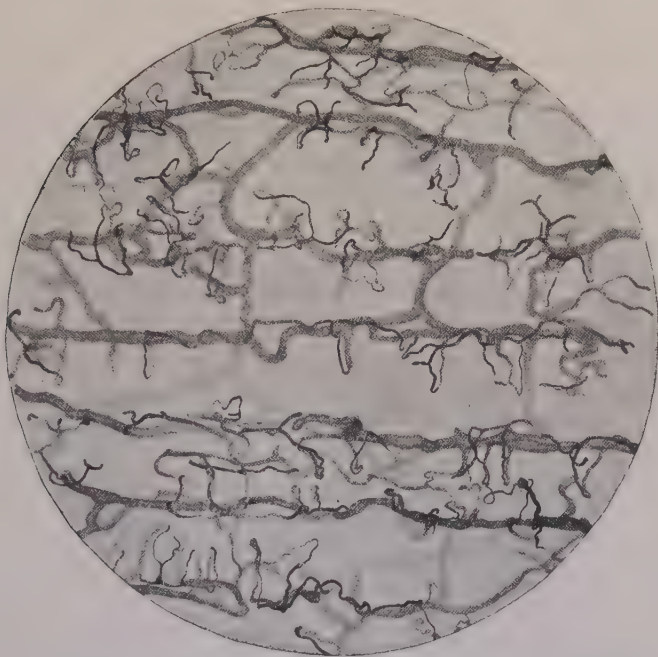


Fig. 11. First subpapillary venous plexus with narrow arterial branches and capillary loops. After Spalteholz.  $\times 41$ .

It is a characteristic feature of the blood vessels of the human skin that there is no very sharp distinction between the smallest arterioles ("capillary arteries," Spalteholz), the capillaries proper, and the venules ("capillary veins"). Lewis (1927, p. 3) introduces the term "minute vessels" of the skin to designate these vessels and distinguish them from the "strong arte-



rioles" and the "deep veins." The main point, which will be discussed in some detail in Lecture IV, is that the venules in the outer part of the cutis are so thin-walled and present such a large total surface that they have practically taken over the normal function of capillaries, viz., the exchange of substances. In the deeper layers of the skin the larger veins are accompanied by numerous smaller vessels branching off from them and returning to them at a lower level and these, of course, fulfil the same capillary function of exchange.

The vascular surfaces available for exchange of substances in the human skin have not been made out. The total surface of the capillaries proper is extremely small, being something between 1 and 2 cm.<sup>2</sup> per cm.<sup>2</sup> of the skin surface. Even when the whole surface of the veins is assumed to be available for exchange the total surface will fall far short of the capillary surface available in muscles, and the average distances from the tissue elements to the vessels will be much larger. This is no doubt the anatomical expression of the fact that the metabolic level of the skin is low and probably much less variable. This point will be referred to again in Lecture XII.

*The capillary system in the intestinal villi.*

Through the columnar epithelium covering the surface of the villi in the small intestine practically the whole of our daily food is transported. Once inside the epithelium of a villus there are two ways open to the absorbed solution of foodstuffs. The dissolved substances can pass either into the network of capillaries below the surface or into the lymph system, represented in each villus by the central lacteal. The distribution of the substances between these two channels must depend to a large extent on the surface develop-



ment of the capillary system and its relation to the epithelial surface of the villus. No real insight into the physiological processes can be obtained unless these surfaces are, at least approximately, made out.

An attempt in this direction was made long ago by Mall (1887), but it cannot be denied that his calculations are rather summary and his results roughly approximate only.

Mall found in the dog's small intestine 16 villi per mm.<sup>2</sup> These are approximately cylindrical, with a height of 0.5 to 0.6 mm. and a diameter of 0.2 to 0.25 mm. I calculate the surface of each villus to be about 0.43 mm.<sup>2</sup>

Mall describes a small artery entering each villus

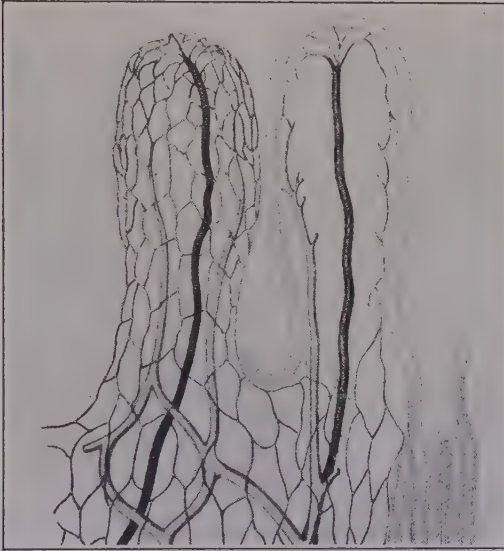


Fig. 12. Villi from the small intestine of dog.  
Height of villus about 0.5 mm. After Mall.

and running right up to the top, where it splits up suddenly into 15 to 20 capillaries which travel down along the internal surface of the epithelium, forming a close-meshed network (Fig. 12). The average diameter of the single capillaries is given as  $8\mu$ . At a height varying between one-third and one-half of the height of the villus, part of the capillary blood is taken up by veins, but the capillary network continues downward, with the difference that there are fewer capillaries and their average diameter is only  $5\mu$ .

According to Mall the capillary system in the upper two-thirds of the villus could be represented by 30 parallel tubes 0.4 mm. long and  $8\mu$  in diameter, and the capillaries in the lower third by 15 tubes of 0.2 mm. length and  $5\mu$  diameter. From these figures the total capillary surface in each villus works out as  $0.35 \text{ mm.}^2$  or 82 per cent of the surface of the epithelium.

On each  $\text{mm.}^2$  of the internal surface of the intestine we find an epithelial surface of the villi amounting to  $7 \text{ mm.}^2$  and a capillary surface of  $5.6 \text{ mm.}^2$

In my laboratory Dr. Vimtrup has made a preliminary study of the quantitative anatomy of the absorbing system in the rabbit's intestine. I shall give only the measurements undertaken on a single villus in the duodenum, shown in Fig. 13. As seen in the figure the villi in this part of the rabbit's intestine are not cylindrical, but have an elliptical transverse section. The capillary system is very irregular compared with the one described by Mall. It is supplied by two small arteries and drained mainly by four veins.

The larger vessels run at some distance from the epithelial surface, but it is of considerable importance from a physiological point of view that almost all the capillaries are practically cemented on to the bases of the epithelial cells.

On a large-scale drawing, on which only the capillaries of one side of a villus have been represented by single lines, the total length of capillaries can easily be

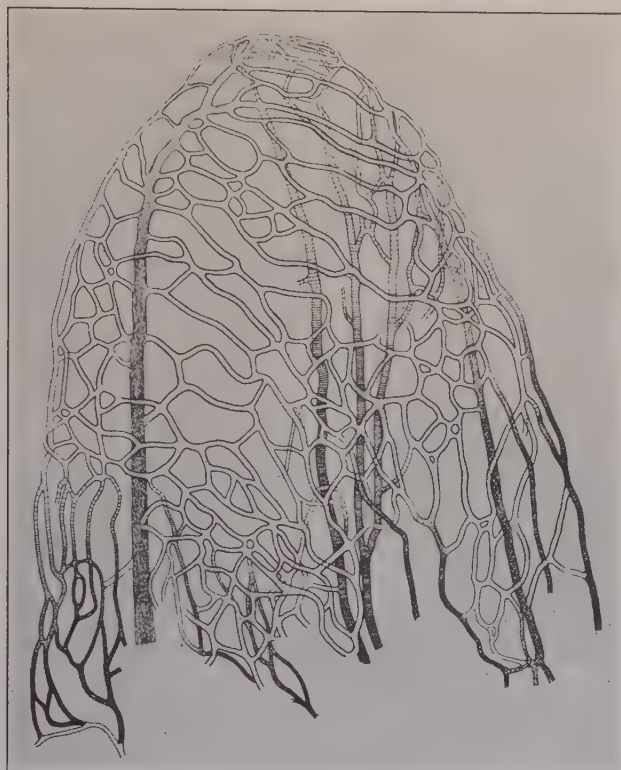


Fig. 13. Villus from small intestine of rabbit, showing capillary network of one side.  $\times 68$ .

determined by running a small measuring wheel along them. On a surface of  $0.84 \text{ mm.}^2$  the total length of capillaries amounted to  $2.47 \text{ mm.}$  The average diameter of each capillary was found in the injected speci-

men to be  $12\mu$ , giving a total surface of  $0.93 \text{ mm.}^2$  or 109 per cent of the epithelial surface. Multiplying the total length of the capillaries by their average diameter, instead of by their circumference, we obtain the projection of capillaries on the epithelial surface as  $0.295 \text{ mm.}^2$ , or 34 per cent, which means that about one-third of the epithelial cells will give off the substances passing through them directly into the capillaries, while two-thirds will deliver them into the intercapillary spaces.

The total surface of one villus works out as  $2.2 \text{ mm.}^2$ , with a capillary surface of  $2.4 \text{ mm.}^2$  and a capillary projection of  $0.75 \text{ mm.}^2$ . With from 6 to 10 (average about 8) villi per  $\text{mm.}^2$  internal surface of the duodenum we find an epithelial surface per  $\text{mm.}^2$  of  $17.6 \text{ mm.}^2$  and a capillary surface of  $19.2 \text{ mm.}^2$ , both of which figures are more than double those found by Mall for the dog.

Some use will be made of these results in the final chapter, where the mechanism of the distribution of absorbed substances between the blood and the lymph will come up for discussion.

*The capillary surfaces available in the glomeruli.*

Vimtrup has made an extremely interesting and valuable quantitative study of the glomerular apparatus of the kidney, results of which have already been utilized by Rehberg in his investigations on kidney function (1926).

Vimtrup has placed at my disposal the following results of countings of the glomeruli, comprising three cases in which every individual glomerulus present in a kidney was counted, while in the others the total number was deduced from countings representing from 3 to 31 per cent of the whole kidney.

Species	Kidney weight	Glomeruli counted	Total number of glomeruli in one kidney
Rat 205 g.	1.05 g.	33,826	33,826
Cat 2.8 kg.		202,813	202,813
Cat, ca. 3 kg.	12 g.	7,342	173,800
Cat, ca. 3 kg.	12 g.	5,586	171,100
Dog 8 kg.	29 g.	126,856	407,150
Dog 12 kg.	38 g.	34,836	507,900
Man, adult, negro		49,565	834,000
Man, child		71,281	900,000
Man, child		955,251	955,251
Man, adult	143 g.	30,374	980,000
Man, adult	165 g.	73,176	1,233,360

An approximate estimate of capillary lengths and surfaces in the human glomerulus is obtained according to Vimtrup, as follows. The glomerulus is spherical

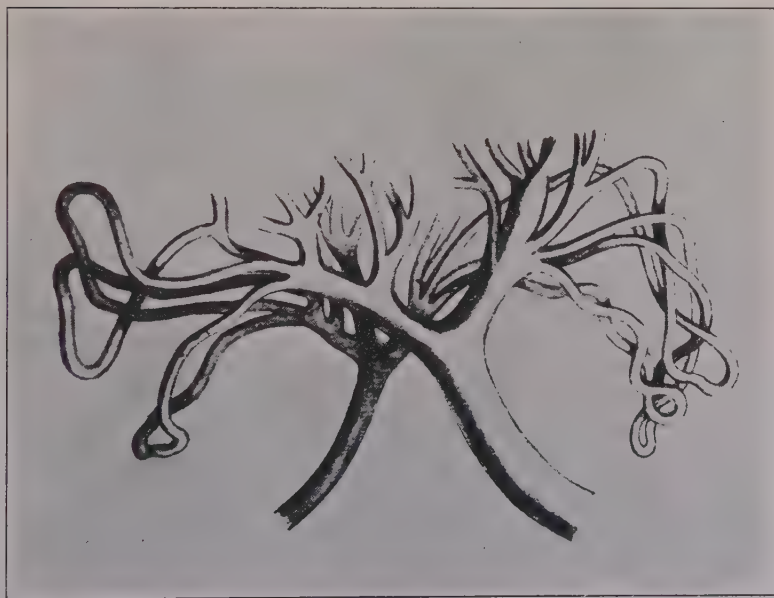


Fig. 14. Division of glomerular vessels. After Vimtrup.



in shape with a diameter of about  $200\mu$ . The volume works out, therefore, at  $0.0042 \text{ mm.}^3$  Vimtrup has found by a histological study, which will be referred to in some detail in a later lecture (p. 97) that the glomerulus is made up of a variable number of capillary loops which do not anastomose, but run a very twisted course from the afferent to the efferent artery as illustrated by the diagram Fig. 14. The number of these loops is on an average about 50, and their length is estimated at about 2.5 times the diameter of the glomerulus or 0.5 mm., giving a total length of capillary in each glomerulus of 25 mm., or for the whole kidney with 1 million glomeruli, 25 kilometers. The average diameter of the capillaries is about  $10\mu$ . The volume of 25 mm. tube with  $10\mu$  diameter works out as  $0.002 \text{ mm.}^3$ , or about  $\frac{1}{2}$  the glomerular volume, which seems reasonable. The available surface is found to be  $0.78 \text{ mm.}^2$  for each glomerulus, or for both kidneys in the human subject about 1.5 square meter, corresponding to a capillary volume of 4 cc.

*The rete mirabile annexed to the oxygen gland in the eel.*

I shall finally describe briefly a very peculiar arrangement of capillaries, a true *rete mirabile*, found in the swim bladder of fishes and presenting the most extreme development of capillary surface known to me.

In the wall of the swim bladder of most fishes there is an oxygen gland, the function of which is to take oxygen from the blood and secrete it into the swim bladder, where the oxygen pressure can become very high. Between the vessels of the oxygen gland and the general circulation of the fish the organ of which I am now speaking is interposed. Its form varies greatly, but its essential structure is the same everywhere, according to Woodland (1911). The description I am

about to give refers especially to the organ of the eel and is represented in the diagram, Fig. 15. The artery supplying the oxygen gland splits up at a definite point into numerous branches, which divide further into an enormous number of parallel capillaries. These capillaries run as straight tubes for a definite distance and then suddenly unite again and form an artery which goes to the gland, where it supplies an ordinary network of arterioles, capillaries, venules, and veins, uniting finally into a single vein, which comes back to the distal end of the arterial rete, splits up just like the artery into straight parallel capillaries, which are intercalated with the most astonishing regularity between the arterial capillaries and which, finally, unite at the proximal end of the rete to form a single vein.

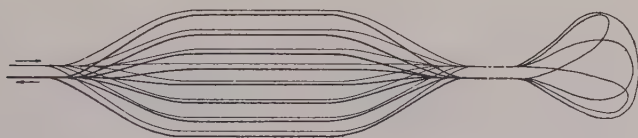


Fig. 15. Diagram of rete mirabile and capillary loops from swim bladder of eel.

When the vessels are cut open between the gland and the rete and cannulas introduced into the proximal artery and vein it is easy to inject the rete with two differently colored gelatines and to obtain preparations which, when cut in suitable sections, will give pictures like the one reproduced in Fig. 16. The arterial capillaries are gray, the venous black, and you will notice how each venous capillary is regularly surrounded by a number of arterial capillaries which are somewhat narrower.

Countings and measurements on such sections have given, in the case of a medium-sized eel, the following

results. There were two parallel retes, each of which had a cross-sectional area of 8.0 sq. mm., while the capillaries of which it was composed had a length of 4 mm., a very great length when it is remembered that the capillaries in muscles, which are otherwise among the longest, are seldom more than  $\frac{1}{2}$  mm. long. The

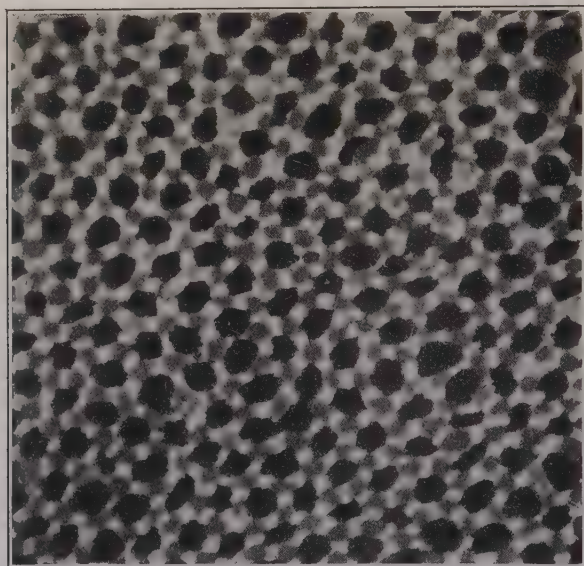


Fig. 16. Transverse section of rete mirabile from swim bladder of eel. Arterial capillaries gray. Venous capillaries black.

total volume of the two capillary systems is, therefore, only 64 cubic millimeters.

What surfaces can it be possible to get into a volume of 64 mm.<sup>3</sup>, the size of a drop of water?

As the capillary system is of the most extraordinary regularity throughout, I have only counted the capillaries in two contiguous narrow rectangles 0.33 mm.

long and 0.033 mm. broad. In the one I found 60 venous and 77 arterial, in the other 60 venous and 81 arterial capillaries, giving a total of 120 venous and 158 arterial in the area of 0.0218 mm.<sup>2</sup>, or per mm.<sup>2</sup> of the cross-section 5,500 venous and 7,250 arterial capillaries. For the whole of both organs this will give 88,000 venous and 116,000 arterial capillaries with aggregate lengths of 352 and 464 meters.

In another area the capillaries were drawn by means of a prism at a high magnification to make out the relative cross-sectional areas of venous and arterial capillaries and of interstitial tissue, including the capillary walls, respectively. The area measured in this way was only 0.00386 mm.<sup>2</sup> It contained 21 venous capillaries occupying an area of 0.00149 mm.<sup>2</sup>, or 38.6 per cent of the whole, and 34 arterial, with an area of 0.00108 mm.<sup>2</sup>, or 28.1 per cent. The interstitial tissue works out to be just 33.3 per cent of the whole. On the basis of the countings given above the area should contain 21 venous and 28 arterial capillaries, a very satisfactory agreement with the numbers 21 and 34 actually found.

Dividing by the average number we find 71 and 39 square microns ( $\mu^2$ ) as the average cross-sectional area of a venous and an arterial capillary, respectively. Taking these cross-sections to be circles, which is, as you will see on Fig. 8, not quite correct, we find the diameters to be respectively, 9.5 and 7.1 $\mu$ , and the circumferences (corrected for their not being circles), 30 and 22.5 $\mu$ , respectively. When these figures are multiplied by the aggregate lengths we obtain the interesting result that the venous and arterial capillary surfaces in these organs are equal, being respectively, 106 and 105 cm.<sup>2</sup>, while the total volume of the venous capillaries is about 25 and that of the arterial about 18 cubic millimeters.

*A plea for the study of quantitative anatomy.*

Most of the measurements and calculations here given have been undertaken because they were directly required for physiological researches, carried out or planned in my laboratory. They are, with the exception of Vimtrup's countings of glomeruli, crude and not very accurate, because the work was quite outside our regular routine, and we did not want to go any farther than just necessary for our immediate purposes. I believe, however, that, when taken up in earnest by competent anatomists, the field of quantitative anatomy will prove to be a rich and fruitful one.

Many determinations of vascular and glandular surfaces are urgently needed as a basis for quantitative physiological work. I shall mention as instances only the active surfaces of various glands and the dimensions and numbers of sarcomeres in muscle fibers. Quite apart, however, from the needs of physiology, I cannot but think that quantitative anatomy will prove a very attractive subject for its own sake, especially when conducted as a comparative science.

## NOTE

<sup>1</sup>In a publication received after the completion of the manuscript Wearn (1928, 1) gives a large number of very careful countings of capillaries from the hearts of rabbits, cats, and man. His figures are much higher than Stoel's. The main direction of capillaries is along the fibers but the oblique and transverse anastomoses are much more numerous than in skeletal muscles, forming a network with meshes 3 to 4 times longer than broad. The number of capillaries per 1,000 muscle fibers is about the same in both ventricles, septum, and papillary muscles in all hearts, namely, from 1,000 to 1,100. In auricles and Purkinje fibers it is lower (500-600). Calculated per sq. mm. transverse section the figures differ more and are for the ventricles of a human heart 5,200-5,700, for the cat about 4,000 (3,943-4,340) and for the rabbit 5,600-6,100.

The existence of anastomoses between the coronary artery and the Thebesian veins makes it very difficult to obtain complete injection except by a special perfusion technique, and I have no doubt that Stoel's injections reported above have been incomplete.



### LECTURE III

#### THE INDEPENDENT CONTRACTILITY OF CAPILLARIES

**I**N the preceding lecture I described the wonderful arrangement of the capillaries and tried to make clear the enormous extent of the surfaces they present for the exchange of substances between the blood and the tissues. I alluded also very briefly to the problem with which we are now confronted: Supposing these facilities for exchange to be necessary and just sufficient to provide for the needs of an organ when that organ is doing work at its maximum rate, how can they avoid being wastefully in excess of the requirements when the organ is absolutely or comparatively at rest?

The generally accepted view of the capillary circulation, at least until a few years ago, was that the capillaries are passive, that blood flows continuously through all of them at rates which are determined by the state of contraction or dilatation of the corresponding arterioles, and that the dilatation of an arteriole will cause a rise of pressure in the corresponding capillaries, which will become passively expanded, to contract again by their own elasticity when the pressure is reduced.

By the varying resistance in the arteries and arterioles the supply of blood to an organ can undoubtedly be regulated in accordance with its requirements, but an increase in current must always be accompanied by

a corresponding increase in capillary pressure, and when the requirements are small the quantity of blood constantly present in a large number of the capillaries would serve no useful purpose. A much more effective distribution would obviously be obtained if the capillaries themselves were contractile, if in a resting organ only a limited number of capillaries, distributed at suitable regular intervals, were kept open so as to admit blood and provide the necessary surface area for the exchange of substances. This hypothetical conception was to me personally the starting point and guide in the experimental study of capillary contractility. Since, however, I was by no means the first to discover or even to demonstrate that capillaries show independent contractility, the most suitable course will be to present the evidence in the order in which it was published.

*The older experiments on capillary contractility.*

In a recent valuable paper Stegemann (1927) has brought together and discussed a number of references to books and papers from the first decades of the nineteenth century, in which observations and experiments are described, very similar to those which now form the basis of the doctrine that capillaries possess independent contractility and play an important part in regulating the supply of blood to the tissue cells.

It should be borne in mind, however, that the physiological and pathological problems of those days were different from ours, and it is only in the light of much later work that the old investigations can be considered as relevant to our problems. The main question then was whether the heart could possibly provide sufficient driving force for the circulation. Those who thought that it could not were seeking for evidence of peripheral propulsive agents, and in doing so they discovered that the small blood vessels, which could be ob-

served under the microscope, had a power to contract and could be stimulated to contraction or dilatation in various ways (Philip, 1804; Philip, 1826; Hastings, 1820). They did not clearly distinguish between capillaries and small arteries, but utilized their observations as evidence to show that the small vessels were able to keep up a flow of blood to a certain extent without the help of the heart.

Their opponents (Wedemeyer, 1828; Johannes Müller, 1834) whether admitting or denying the contractility of smaller arteries rightly contended that the contractions observed could only effect a reduction of blood flow through the parts concerned.

Wedemeyer and also Döllinger (1821) having seen capillary paths opening up under the microscope (comp. Fig. 22, p. 67) consider that most capillaries have no organized walls whatever, but are formed by the blood finding its way from the arteries to the veins, and assume that the large increases in blood content, observed for instance in inflammation, are due to the attraction ("Blutgefühl") exerted by the inflamed tissue.

It is a curious fact that while the observations of this period relating to capillaries and capillary circulation seem to have been quickly and completely forgotten the theories concerning "peripheral hearts" and "Blutgefühl" have been cropping up again and again and are even now seriously discussed and refuted (see p. 230).

While working on the problem of inflammation, brought about experimentally in the web of the frog, Lister (1858) observed that capillaries could become enormously dilated, but he states definitely that in his opinion the dilatation is brought about by the increased pressure brought to bear on their walls by the dilatation of arteries, and the first to notice an

independent contractility of capillaries was, so far as I have been able to make out, Stricker (1865), working on the excised nictitating membrane of the frog, in which he observed irregular spontaneous contractions and relaxations of single capillaries. He tried to evoke contractions by suitable stimuli, but it was only very occasionally that he was successful in this. Since the membrane was excised the blood pressure could have nothing to do with the movements observed, but on the other hand the apparent fitfulness of these, and the

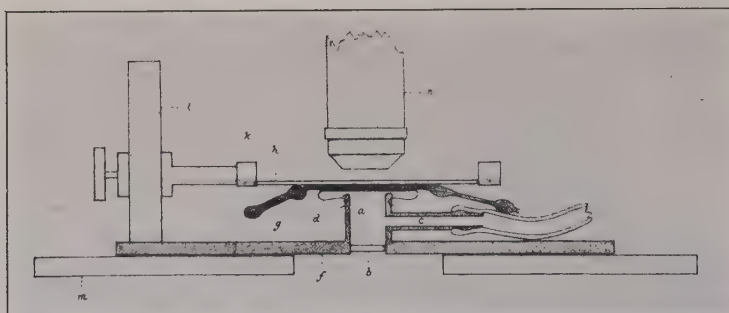
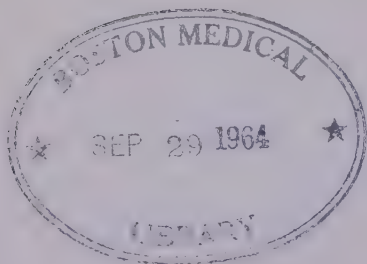


Fig. 17. Roy and Brown's apparatus for measuring capillary pressure.

fact that they were observed in conditions which could not be regarded as physiological, did not inspire confidence. His statements were challenged by several authors (Cohnheim, 1867), and, though they were confirmed and somewhat extended by others, no very definite evidence was forthcoming until the publication of the beautiful researches of Roy and Graham Brown (1879). These authors constructed the ingenious apparatus shown in Fig. 17 for applying pressure to a transparent tissue, such as the web of the frog, and thereby measuring the blood pressure in the vessels of that tissue. The apparatus consists of a chamber (a)



closed below by a glass plate (*b*) and above by a delicate and very flexible, but inelastic, membrane (*d*). The air pressure inside the chamber can be raised to any desired height through the tube (*c*) and measured by a suitable manometer. The transparent tissue to be studied is arranged on top of the membrane and pressed up against the adjustable cover-glass (*h*).

In their experiments with this apparatus Roy and Brown found, as one would expect, that the pressure which was just sufficient to cause the collapse of one capillary might be insufficient for its immediate neighbors, but they observed further that the pressure relations were constantly changing. "If, for example, we take a curarized frog, and having arranged the compressing apparatus in the manner above described, and having sketched roughly the position and relations of the various capillaries which can be seen in the field of the microscope, we mark on our drawing the order in which the capillaries cease to admit the passage of blood-corpuscles on gradually increasing the extra-capillary pressure; if, having done this, we lower the pressure to which the portion of tissue is subjected to 0, and, after leaving everything untouched for, say, half an hour, and again investigate the order in which the capillary vessels of the same part become impervious on raising slowly the applied pressure, we usually find that there is a more or less marked difference in this respect between the two observations. Occasionally it is found that those capillary vessels which closed in the one observation with a relatively low pressure on their exterior are, in the other observation, those which remain longest pervious to the blood flow; and this, although every possible precaution has been taken to insure that the conditions should remain unchanged." These changes they rightly explain as due to spontaneous changes in the caliber of the single



capillaries, and in some cases they have been able to measure these changes directly. They find, further, that an extracapillary pressure which is just insufficient to bring about the total collapse of a capillary has no appreciable influence upon its diameter; that is, it may cause a shrinkage amounting to at most 15 per cent, which shows that elastic expansion by inside pressure does not play more than a comparatively insignificant part in determining the diameter of capillaries, and this is even more forcibly brought out by the fact that a sudden diminution of the internal pressure to about 0 does not cause more than a slight contraction of normal or even of exceptionally dilated capillaries.

During the later years of the nineteenth century, the doctrine of independent capillary contractility was, as far as I have been able to make out, tacitly or openly accepted by many pathologists and clinicians, who are certainly very often brought face to face with cases of hyperemia which are, to say the least, difficult to understand on the basis of any other theory; but the general attitude of physiologists working on circulation problems by means of blood pressure, plethysmographic, and other methods was, during the same period, very skeptical and remained so in spite of the fresh, and to my mind conclusive, evidence on the subject brought to light by Steinach and Kahn (1903).

Steinach and Kahn again examined excised tissue, the nictitating and other membranes from the frog and the omentum from young cats. By suitable electrical stimulation they were able to induce contractions of true capillaries in all these tissues. The contractions were sometimes sharply localized, sometimes extending over long distances. By varying the intensity of the stimuli they could determine the degree of contraction from a just perceptible narrowing to a complete closing of the capillary. They found that the

stimuli acted after a latent period of some seconds, that the contracted capillaries dilated slowly after cessation of the stimulation, and that the same capillary could be made to contract repeatedly up to 10 or even 20 times.

When the nervous connection of the nictitating membrane with the body was kept intact, though the natural circulation through it was abolished, they were able to induce contractions by stimulating the dorsal sympathetic. In this case the time of latency was prolonged and the capillaries responded only after the arteries had been brought to contraction by the same stimulus.

It seems very strange that the experiments of Steinach and Kahn did not arouse any active interest in the physiology of capillaries and were on the whole disregarded by physiologists, though their results were incorporated in one physiological textbook of repute, viz., Tigerstedt's *Lehrbuch der Physiologie*.

In the following years, until 1917, changes in the diameter of capillaries, which appeared to be independent of the arterial blood pressure, were observed and described by some authors, but the real significance of the facts observed was not as a rule grasped,<sup>1</sup> and generally the sources of error inherent in the microscopic observation of capillaries were not recognized or properly guarded against.

#### *The modern study of capillary contractility.*

In 1917 Ebbecke published his paper on the local vasomotor reactions of the skin and internal organs, the outcome of several years' careful observation and experimentation and deep thinking, and this publication marks the beginning of a new epoch in the study of the capillaries, because he was the first to recognize clearly the full significance of the facts brought to light. Ebbecke's paper contains a wealth of informa-

tion, and I shall have to refer to it very often in the course of these lectures, but at this stage I am concerned only with the evidence of independence between the reactions of capillaries and arteries.

Ebbecke describes the following experiment on frogs which were curarized and kept moist, while the web of one foot was pinned out and allowed to dry up slowly at rates which could be conveniently varied. He finds that at first the circulation is very slow, the arteries narrow, and many capillaries completely closed, while others allow the passage from time to time of a single red corpuscle. During the first half hour the arteries dilate, a number of new capillaries appear, and the current of blood becomes very rapid through all the vessels. So far the observations could be taken to favor the view that the capillaries become passively distended by the blood pressure, but at this stage the arteries begin to contract again and become gradually very narrow, while the capillaries become more and more dilated, and the number of visible capillaries is further increased until, finally, it is three to four times the initial. This opening up and dilatation must necessarily be independent of the internal capillary pressure, which at this time is very low.

Ebbecke points out the fact that redness or paleness of the human skin depends upon the quantity of blood *present* in the cutaneous capillaries and venules, that is, upon their state of dilatation or contraction, while the temperature of the skin is chiefly dependent on the rate of blood flow through the skin, and he goes on to show that the skin of the hand, for instance, may be very warm, without being red, while the action of cold may produce a state of pronounced hyperemia characterized by a bluish color, which indicates that the flow of blood is so slow that its oxygen is used up to an unusual extent. Ebbecke concludes that in the warm, pale

hand we have dilated arteries and arterioles without dilated capillaries and in the cold, blue hand the arteries are strongly contracted and the capillaries (and venules) dilated.

In 1917 a paper was also published by Cotton, Slade, and Lewis, in which important evidence is brought forward to show that the capillaries of the human skin are able to contract and dilate independently of the arterioles. These authors studied the dermographic reactions of the human skin, of which I shall have more to say later in the course of these lectures.

A slight stroke along the skin with a blunt point produces in most individuals a white line, while a heavy stroke produces a red line which may be bordered by white. These reactions take place after a time of latency of several seconds, they reach a maximum in half a minute or more and fade away after several minutes; their borders are very sharp and correspond closely to the area directly stimulated. Cotton, Slade, and Lewis are of the opinion that the sharp definition of the white and red bands suggests their origin as capillary reactions, since reactions of arterioles must result in an irregular border line, but the description given in my first chapter of the vascular system of the skin shows that the meshes of arterioles and venules are small enough to render such a conclusion invalid.

They obtained very definite evidence, however, by studying the reactions after suddenly cutting off the blood supply to an arm by means of a sphygmomanometer armlet in which the pressure was raised well above the arterial. When the blood had become completely stagnant they still succeeded in getting quite definite reactions after the usual period of latency, and they maintain rightly that in this case the very vessels, which by their content of blood are responsible for the color of the skin, must have contracted or dilated.

There can be no doubt that these vessels are the capillaries and venules.

They compare the white tache after gentle stroking to the whitening produced mechanically by gentle pressure. The latter begins to suffuse immediately when the pressure is released and disappears completely in a few seconds, because blood runs into the open capillaries from all sides. The former develops *after* the stroking and is maintained for a comparatively long time. It must be impossible, therefore, for the blood to get into the vessels responsible for the color: they must be actively closed.

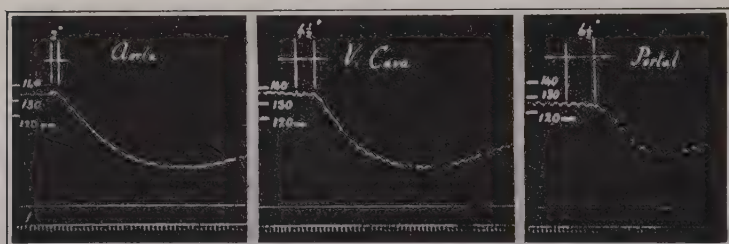


Fig. 18. Effects of intra-aortic, intracaval, and intraportal injections of 0.01 mgm. histamine. After Dale and Richards.

The paper of Dale and Richards (1918) contains a detailed and very close-reasoned comparison between the actions of three "depressor" drugs, leading up to the conclusion that one of them must produce a relaxation in the tone of arterial smooth muscle, while the other two must produce relaxation of the capillary wall. I propose to state the reasoning of Dale and Richards in some detail, not only because it is an exceptionally beautiful example of physiological analysis, but also because it serves to expose the inherent fallacy of the classification of substances acting on the



circulation as either "pressor" or "depressor," a point on which I shall have more to say hereafter.

The analysis takes its starting point from the observation that in carnivorous mammals infinitesimal doses of either histamine, adrenaline, or acetyl-choline injected into the blood stream produce an evanescent fall of arterial blood pressure.

In larger doses, on the other hand, acetyl-choline has a pure "depressor" action, which can be ascribed to dilatation of arteries, adrenaline shows the well-known "pressor" effect, due to contraction of arteries, and histamine induces in the animals which are susceptible to its action the characteristic symptoms of shock. Dale and Richards show first, by measuring the time of latency between the moment of injection of a small dose of histamine and the onset of the fall of blood pressure, that this drug, like the others, acts chiefly on the peripheral vessels of the systemic circulation, the latent period being much shorter with intra-aortic than with intracaval or intraportal injections. They go on to examine the effect of small doses of the substances studied by simultaneous arterial blood pressure records and plethysmograph records of the volume changes of selected parts, usually one of the hind legs. On animals with intact nerves they find that acetyl-choline will always produce dilatation, while the other two drugs are more variable in their action, sometimes producing dilatation, sometimes contraction. When the nerves to the limb are cut, an increase in volume results, due, chiefly, at least, to a dilatation of the arteries, and when the three substances are tested under these conditions the dilator effect of acetyl-choline is found to be diminished for the time being and to return only as the arterial tone is regained, while a considerable dilator response to either histamine or adrenaline remains throughout (Fig. 19).

This suggests that the site of action of these two drugs is different from that of acetyl-choline. Then, again, if a leg, and preferably a denervated leg, which can be relied on to give constant dilatation on injection of any

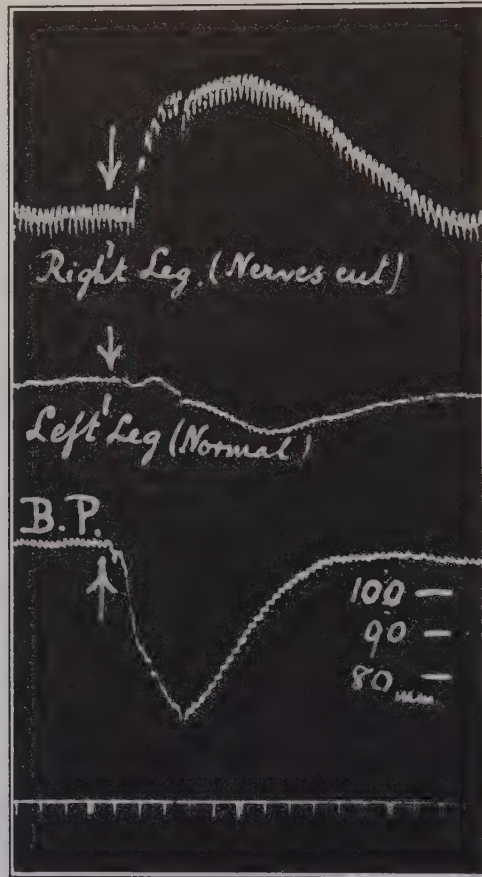


Fig. 19. Effect of 0.01 mgm. histamine. Volumes of leg with nerves freshly cut and of normal leg; blood pressure. Time markings, 10 seconds. After Dale and Richards.

of the substances, is made anemic by occlusion of the vessels, a great increase in volume takes place when the blood is again admitted, and in this condition the effect of either histamine or acetyl-choline is a *decrease* of volume due to a mere passive following of the concomitant reduction of general blood pressure. As the swelling passes off the normal dilator effects return, but at different rates for the three substances—a stage being found in which acetyl-choline produces dilatation, while histamine still shows the passive shrinkage resulting from fall of blood pressure. This is a further indication of the difference in the site of action of these two drugs.

It was already well known that acetyl-choline produces its depressor effects by relaxation of arterial tone. We must, therefore, conclude that the dilator effect of histamine and adrenaline must be localized in another part of the circulatory system and probably in the capillaries.

This conclusion is supported by a series of perfusion experiments. It was found that while the dilator action of acetyl-choline can easily be demonstrated plethysmographically on a limb perfused with oxygenated gum Ringer, so long as the arteries retain their tone, and will be accompanied by a large increase in outflow from the perfused limb, the dilator action of histamine would only take place when an adequate supply of oxygen was secured by the addition of red corpuscles to the perfusion fluid, and when this contained a sufficient amount of adrenaline (1 part in a million to 1 in 10 millions). Under such conditions a large increase in volume of the perfused limb will be accompanied by only a small increase in flow.

Special perfusion experiments on a preparation of the superior mesenteric artery and all its ramifications up to the line where they enter the intestines showed

conclusively that the effect of histamine on arteries is under all conditions a contraction, diminishing the rate of outflow.

The conclusion drawn from these perfusion experiments is again that the dilator action of histamine must be exercised beyond the arteries on the capillaries and can be exercised only when the tone of these vessels is kept up by a sufficient supply of oxygen and by the presence of a tonic substance, such as adrenaline in suitable concentration.

Dale and Richards further strengthen their conclusion by some interesting and important evidence of a more direct nature. On cats with unpigmented feet they have made the observation that the pads of the foot in a denervated leg become distinctly warmer, but at the same time paler, than the pads of the normal foot. By immersing each foot in 10 cc. of cold water and noting the increase in temperature of the water they have shown conclusively that there is through the denervated foot a more rapid flow of blood, and that the pale color must, therefore, mean that the capillaries are contracted in spite of the increased pressure due to arterial dilatation. In such a cat, injection of 0.01 mg. histamine causes a definite flush (dilatation of capillaries) in the denervated foot, while acetyl-choline has no distinct effect on the color.

When a pledget of cotton wool soaked in 0.1 per cent histamine is applied locally to the surface of the cat's pancreas, in which there are no vessels of such a size as to be visible to the naked eye, a distinct red flush is produced in 10 to 20 seconds, showing that the minute vessels are dilated.

Dale and Richards are careful to point out that the evidence which they have brought forward does not warrant any sharp distinction between the capillaries proper and the smallest arteries, and it could, I think,

be argued that the reactions which they have observed might be reactions of arterioles, which would imply, however, that these should behave quite differently from those larger arterial branches which are visible to the naked eye.

My own first contribution to the problem of capillary contractility was published in Danish in 1918, about a month after Dale and Richards' paper, and somewhat later appeared in the British *Journal of Physiology* (1919). It was undertaken to test the hypothesis of a regulation of the supply of blood to muscles through the opening and closing of individual capillaries. I found it possible to observe at least the superficial capillaries of muscles both in the frog and in mammals through a binocular microscope, using strong reflected light as a source of illumination. Resting muscles observed in this way are usually quite pale, and the microscope reveals only a few capillaries at fairly regular intervals. These capillaries are so narrow that red corpuscles can pass through only at a slow rate and with a change of form from the ordinary flat disks to elongated sausages. When the muscle under observation is stimulated to contractions a large number of capillaries become visible and dilated, and the rate of circulation through them is greatly increased. When the stimulus has lasted only a few seconds the circulation returns in some minutes to the resting state; the capillaries become narrower and most of them are emptied completely, while a small number remain open. Since capillaries, even in a group fed by the same arteriole, do not all behave in the same way, the changes obviously cannot be due to arterial pressure changes.

In resting muscles of the frog the average distance between open capillaries, observed simultaneously through the microscope, was estimated at 200 to 800 $\mu$ ,



but after contractions this could be reduced to 70 or 60 $\mu$ . In the guinea pig average distances of about 200 $\mu$  could be observed during rest. The exposure to the air and the strong light always increased the circulation, and it was often possible to see the circulation begin in one capillary after another.

It might be argued that the observations here recorded could be explained as the results of dilatation of arterioles alone, on the assumption that the capillary paths offer various degrees of resistance, that a few are opened by a low pressure, while the majority of capillaries belonging to an arteriole require a higher pressure and are opened only when that arteriole is dilated. Such an assumption would involve as a consequence that a reduction of the pressure to 0 by cutting the artery must produce an elastic contraction and emptying of the postulated high-pressure capillaries. Numerous observations have shown that all the capillaries may remain open when a piece of muscle is cut out after stimulation.

The measurement of distances between open capillaries made upon living specimens could not, of course, be very accurate, and the degree of regularity of their distribution could not be satisfactorily made out by simple inspection. I had, therefore, to try and devise a method by which the state of the vessels at any given moment could be studied after fixation. This I succeeded in doing by injecting an Indian ink solution, dialyzed against a Ringer solution to make it isotonic with the blood and deprive it of the toxic substances present in the commercial preparation. When a suitable quantity of Indian ink is introduced into the circulation of a living anesthetized animal it is evenly mixed with the blood, and if the animal is suddenly killed by stopping the circulation a few minutes later, and prepa-

rations are made from the muscles and other organs, these show the capillaries which were open at the time.

On frogs I found by this method that there were large differences between different organs in the number of open capillaries. The skin, liver, and brain were always well injected, with all, or nearly all, capillaries open. The tongue was generally white and nearly bloodless, when not stimulated before being removed. The empty stomach and intestines had only a small number of open capillaries. The injection of muscles was variable, but in most of the resting muscles few capillaries only were open, while muscles which had been tetanized before stopping the circulation were almost black from the large number of injected capillaries. Countings of the open capillaries on transverse sections of muscles injected vitally in this way demonstrated the fairly regular distribution of open capillaries. In stimulated muscles I counted in one case of an extensor tarsi muscle 195 capillaries per mm.<sup>2</sup>, while the corresponding unstimulated muscle from the other leg had so few that an accurate count could not be obtained. There were certainly not more than 5 in a mm.<sup>2</sup> Other resting muscles showed higher numbers, however, especially the rectus abdominis muscle, which had also been observed in the living state to be always well supplied with blood, and three countings from which gave, respectively, 115, 155, and 180 open capillaries per mm.<sup>2</sup>, which is from 30 to 40 per cent of the total number. On guinea pigs considerable differences were observed between the degrees of vascularization of different resting muscles, probably connected with the length of time since they had been in activity. Some flat muscles were examined provisionally in the fresh state by counting under a low power the capillaries visible in a single field without using the vertical adjustment. When the positions of capillaries along the

eyepiece micrometer are noted, a fairly good idea of the regularity of their distribution can be obtained. I give some instances of such countings on muscles from the abdominal wall and from the diaphragm of the guinea pig.

		Capillaries at scale divisions															
Muscle from abdominal	}	10	13	16	21	24	28	31	33	35	38	44	50				
wall upper layer:		55	61	65	67	72	75	78									
1 division = 21.8 $\mu$ .																	

Largest distance 6 divisions, smallest 2, average  $3.8 = 83\mu$ .

Diaphragm:	20	22	23	25	27	30	32	35	37	39	41	42	44	46	48	50
1 division = $8.8\mu$ .	51	53	55	57	59	62	64	66	68	70	71	73	75	77	79	81

Largest distance 3, smallest 1, average  $1.96 = 17\mu$ .

The first muscle had been at rest, but the diaphragm, being the chief respiratory muscle, had been working vigorously up to the death of the animal. Several

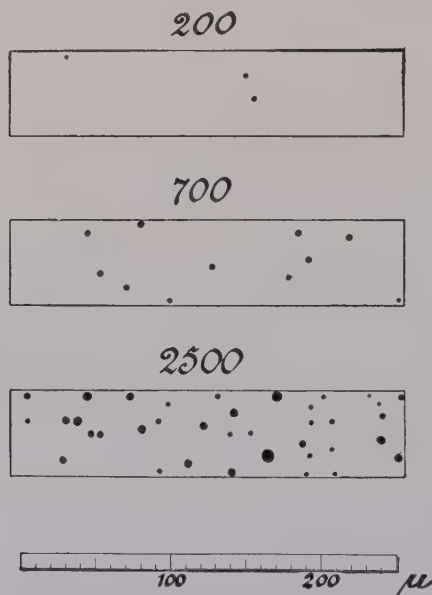


Fig. 20. Preparations from vitally injected muscles from guinea pig. Optical transverse sections.

countings of optical transverse sections of the same muscles, which is, of course, a more accurate method, gave for the muscle from the abdominal wall the figures 86, 70, 92 capillaries per mm.<sup>2</sup>, corresponding to a distance of  $125\mu$  between them, and for the diaphragm 2,700, 2,550, 2,450 capillaries per mm.<sup>2</sup>, corresponding to a distance of  $18\mu$ .

Fig. 20 shows equal optical sections from three different muscles with the number of capillaries per sq. mm. as noted. In this figure the approximate diameters of the open capillaries are also indicated, and it should be noted that in the working muscle they vary considerably, while in the resting muscle, with 200 open capillaries, these are extremely narrow. The table gives a number of measurements in micromillimeters. When it is remembered that the red corpuscles of the frog are on an average  $22\mu$  long,  $15\mu$  broad, and  $4\mu$  thick in the middle, while those of the guinea pig are  $7.2\mu$  in diameter and about  $2\mu$  thick, it seems almost incredible that they can pass through capillaries of the smallest dimensions given and even that they can pass through the average open capillaries of resting muscles. That they do so can be easily observed in the living muscles, as mentioned in the first of these lectures.

Frog, m. sartorius.				Guinea pig.			
Resting		Stimulated		Abdominal wall		Diaphragm	
7.3	5.2	7.2	7.4	2.2	4.0	4.4	4.2
3.5	2.4	7.6	7.3	4.1	1.8	4.8	4.0
2.5	4.1	6.0	8.0	3.0	3.8	7.4	2.8
10.6	3.6	7.0	4.3	4.2	3.0	8.2	5.8
2.9	4.3	7.6	4.2	4.5	3.5	3.3	7.4
4.6	2.1	4.1	6.7	2.7	6.5	6.7	10.4
5.6	2.7	9.6	6.7	3.7	3.1	2.2	3.5
5.5	3.3	5.5	10.7	2.5	2.9	4.2	4.6
4.0	2.9	7.4	5.6	2.9	5.0	4.7	5.5
4.4	4.0	4.9	7.0	2.8	3.3	3.1	5.4
Average,							
$4.3\mu$		$6.8\mu$		$3.5\mu$		$5.0\mu$	

I have said enough, I believe, to make it abundantly clear that capillaries are not merely passively distended by arterial blood pressure, but possess a tone of their own and may show contractions and relaxations independently of the corresponding reactions in the arteries. I think it right, however, to record one more experiment which demonstrates in a crucial man-

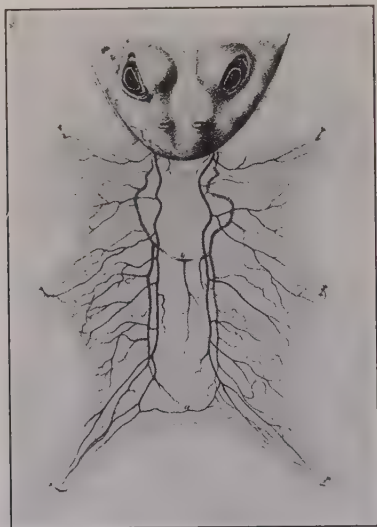


Fig. 21. The frog's tongue pinned out for microscopic examination.  
Natural size.

ner that the whole length of a capillary from an arteriole to a venule can be contractile, that it cannot, when contracted, be forced open by the available arterial pressure, but can be made, by suitable stimulation, to relax and open up to a pressure which is much lower.

The lower surface of the frog's tongue is covered by a smooth mucous membrane and, when suitably



stretched, shows a very wide-meshed network of capillaries, small arteries, and veins. While in the mouth the tongue is usually pale and almost bloodless. When the tongue of narcotized frog is pinned out, as shown in Fig. 21, it becomes stimulated by the process and a large number of vessels appear. When the tongue is left to itself in a moist atmosphere the vessels contract again, the tongue becomes pale and many of the capil-

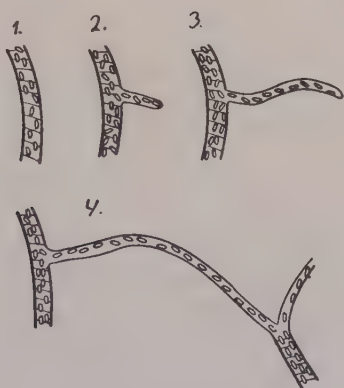


Fig. 22. Opening up of a capillary by repeated weak stimulation.

laries are completely closed and cannot be seen at all with the magnification practicable with the binocular erecting microscope. If the surface of the tongue is now very lightly scratched with a hair or a fine glass needle along a small vein (Fig. 22, 1) a reaction can be obtained like that shown in Fig. 22, 2. A small branch opens up and is filled with blood, which becomes stagnant. By continuing the stimulus in front of the column of stagnant blood the relaxation is carried further (Fig. 22, 3), the blood flows slowly on in the direction from the vein and at last connection is established

with an arteriole, resulting, of course, in a sudden onset of current in the opposite direction, toward the vein.

*The effect of internal pressure on the caliber of capillaries.*

According to the old conceptions regarding the regulating mechanism of the circulation, the arteries and arterioles were almost exclusively responsible for the state of dilatation of capillaries which were opened up by the pressure transmitted to them when the arterial vessels dilated. According to our new conception the caliber of capillaries is mainly determined by their own tonus, but they must, of course, be affected also to a certain extent by the pressure of the blood upon their walls.

It is worthy of note that the direct effect of pressure upon the walls of capillaries is normally very small or even negligible. Ordinarily the pressure which can be brought to bear upon capillaries by dilatation of arterioles is but low, because the integral transverse section of capillary beds and their veins is large compared with that of normal or even greatly dilated arterioles, and we find further that most capillaries are able to withstand a considerable pressure without giving way.

These points will be discussed in some detail in later lectures and here I shall only give a few examples revealed by the preliminary study of capillary circulation which has been the subject of this lecture.

When the main artery supplying one side of a frog's tongue is completely blocked before an area is stimulated no visible dilatation takes place until the blood is admitted. When the arterial pressure is greatly diminished by compression of the artery the dilatation after mechanical stimulation will take place slowly, and after the opening of the artery a further slight dilatation

will take place and a few capillaries may be opened up which had up to that time remained closed.

In the frog's web the arteries can be brought to dilatation by the application of a drop of acetyl-choline and to a partial contraction by adrenaline, and when the capillaries are watched during these changes the effect upon their caliber is seen to be very slight and sometimes scarcely noticeable. When the femoral artery is blocked completely there is a general contraction of the capillaries in the web amounting to some 20 per cent (Oinuma, 1924).

When muscles, especially those of mammals and fishes, are artificially injected in the fresh state it is found to be very difficult to obtain a complete injection, and microscopic examination of some of the injected specimens has revealed the fact that of the number of capillaries supplied by the same arteriole a minority only have become injected, in spite of the high pressure employed.

The power to resist an internal pressure is developed to a very different degree in the capillaries of different tissues. It appears to be very high in muscles and comparatively low in the frog's skin and web. The cutaneous capillaries and venules in the arm of man become somewhat dilated when the venous pressure is raised by means of a Recklinghausen cuff to about 30 cm. water pressure. They appear to resist the pressure for some minutes before they give way.

#### NOTE

<sup>1</sup>The experiments of a single author from this period, Heubner (1907), will be referred to in a following lecture.

## LECTURE IV

### THE STRUCTURE OF THE CAPILLARY WALL

HAVING established the fact of independent capillary contractility we are again face to face with a problem, this time mainly histological: By what means can the contractions be carried out? If we turn to the histological textbooks for information we get a rather disappointing reply. The walls of the arterioles and small veins consist generally of three histologically different layers, viz., an inner tube made up of flat polygonal endothelial cells, an outer coat of connective tissue fibers, containing cells, and a middle portion consisting of one or more layers of smooth, ring-shaped muscle cells. When we approach the capillaries the outer coat first disappears, the muscle fibers become fewer in number and do not form a continuous layer, and finally we have left only the endothelial tube. This is built up of very thin cells of a polygonal or usually of an elongated rhomboidal shape, compared by Stöhr to steel pens pointed at both ends. The cells are cemented together at their edges to form a completely closed tube. The delimitation of the cells can be made very distinctly visible microscopically by treatment with nitrate of silver and subsequent reduction (Fig. 23). The cement will then show up as black lines. By suitable staining each endothelial cell can be shown to possess a nucleus, generally of an oval form and projecting somewhat above the internal as well as on the external surface of the cell.

A structure like this makes it easier to understand why the majority of physiologists have for a long time declined to accept any evidence for capillary contractility, and leads, when the evidence becomes overwhelming, to the assumption of a mechanism of contractility entirely different from that possessed by the larger vessels. The mechanism assumed, I believe, by

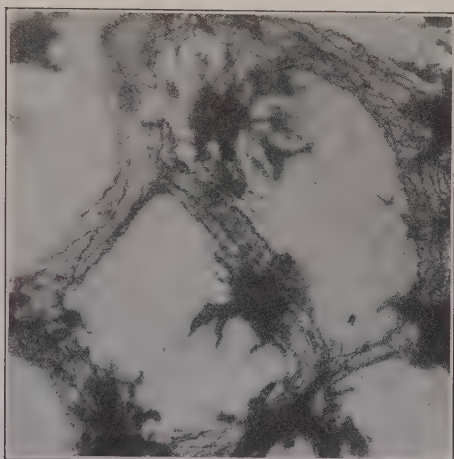


Fig. 23. Capillaries from frog's web. Border lines of endothelial cells silvered. Black pigment cells.  $\times 300$ .

most of the physiologists who have observed the contractility for themselves (Hooker, 1920), was suggested by Stricker (1876) on the basis of his observation that the outside diameter of capillaries did not become appreciably altered, even when the lumen was greatly diminished. This observation leads almost unavoidably to the conclusion that the decrease in internal diameter must be brought about by a swelling of the protoplasm; that, in other words, osmotic or



imbibition processes are responsible for the variations in the internal diameter of capillaries.

*The existence of contractile cells in the capillary wall.*

There exists, however, another and very different conception regarding the contractile mechanism of capillaries.

A few years after Stricker's first communication, Rouget (1873), who had independently observed the contractility of capillaries in young tadpoles, studied histologically the capillaries in the hyaloid membrane of the frog's eye and found on the outside of the endothelial tubes certain oblong nuclei, arranged in the direction of the tube and surrounded by a zone of protoplasm with ramified elongations which embrace the capillary in a number of places like so many hoops. In a second communication (1879) he states having observed these cells also on the living capillaries of young newt larvae and having seen them contract.

He takes them to be related to the smooth muscle cells of larger vessels.

Rouget's papers were practically completely disregarded and soon, as it seems, completely forgotten. Nearly thirty years later Sigmund Mayer (1902) rediscovered the branched cells on the capillaries of the hyaloid membrane and also on those of the intestine of amphibia and stated that a continuous series of cells of intermediate form could be demonstrated between these and the normal spindle-shaped cells of the arterial muscular coat. It did not inspire confidence, however, that these interesting structures could be made visible only by vital staining with methylene blue, and even then only occasionally, and the further statement, that a system of branched cells, similar to that on capillaries, or even isolated cells of the same kind, could occasionally be found where no capillaries

could be detected, could not fail to arouse the suspicion that they had no real physiological connection with capillaries. The suspicious attitude of histologists was probably further strengthened by the fact that, although Mayer concluded his preliminary paper by the announcement that a detailed publication, accompanied by figures, was to appear shortly, this promise was never fulfilled.

Mayer's paper gave, however, the immediate impulse for the physiological investigations undertaken by Steinach and Kahn, and although these authors have not made any positive contribution to the histological problem, they have shown conclusively by measurements of dilated and contracted capillaries that the rival theory of capillary "contractility" by imbibition of the endothelial cells cannot be correct, since they find that the outside diameter of contracting capillaries, far from remaining constant or even increasing, as that theory demands, is very considerably diminished. They give a number of examples, of which I reproduce a few.

Outside diameter of capillaries in frogs'  
nictitating membrane

Dilated	Contracted	Lumen when contracted
$\mu$	$\mu$	
26	12	still open
24	7	closed
22	6	very narrow
19	3	closed

These observations have been repeatedly verified in my laboratory, and the situation shortly before the first edition of these lectures appeared was the following: Of the two theories brought forward to explain the capillary contractility, one—the imbibition theory—was untenable for physical reasons, while the existence of the histological elements demanded by the other

was, to say the least, extremely doubtful. Evidently a solution of this difficulty was essential for a successful attack on the many physiological problems connected with capillary contractility. If the contractility were due to muscles, the innervation, for instance, or the reactions to stimuli must be supposed to be very different from what one would expect if it were due to imbibition processes in the endothelial cells or to a mechanism as yet undiscovered. I, therefore, asked a young histologist, Dr. Vimtrup, to take up the problem, and he reached very definite conclusions (1922) regarding the existence of muscle cells in the capillary wall.

Utilizing the fact that it is possible to bring about experimentally in certain tissues, and notably in the muscles and mucous membrane of the tongue of the frog, any desired degree of capillary contraction and dilatation, Vimtrup has examined sections from such tongues suitably fixed and stained. On the outside of the capillaries he finds certain nuclei slightly, but distinctly, different from the ordinary endothelial nuclei. The form of these nuclei varies with the state of contraction of the capillary. On a dilated capillary they are broad and very thin; by contraction they become narrower and thicker, their cross-section approaching the form of a circle.

The protoplasm belonging to these nuclei is very difficult to stain by ordinary histological methods though Vimtrup has succeeded in obtaining a fairly good staining with iron trioxymatein. Even when stained the protoplasmic structure can only be observed by means of high power immersion lenses, and very good illumination, which must often be made eccentric, is necessary.

The best results were obtained by supravital staining with methylene blue, the method first employed for

this purpose by Mayer and further elaborated by Vimtrup. The application of this method is limited, however, to thin membranes which can be examined in toto without being cut into sections, such as the web, bladder, and nictitating membrane of frogs. The general results obtained by Vimtrup are as follows.

On a dilated capillary the protoplasm surrounds the nucleus as a continuous layer on the capillary wall, but it diminishes in thickness toward the periphery, which is very irregular and sends out a number of very fine branches along and especially around the capillary wall. The branches show at their base a definitely triangular cross-section, but soon become flat. Sometimes they become broader and divide, but the ends are always very thin and pointed. Most of the branches lie athwart the capillary and are of such length that they reach those from the other side. Some of the branches, however, run along the capillary, and both the protoplasm and the nucleus are, as a rule, stretched in this direction. From the ends of the continuous protoplasm a broad branch usually runs along the capillary, giving off very fine secondary branches encircling the vessel. The distance from the last of these branches to the center of the nucleus can, in large cells, amount to about  $100\mu$ .

On a capillary of normal diameter, which will allow the red corpuscles to pass without much deformation, the appearance of the protoplasm is rather different. The continuous mass around the nucleus is distinctly thicker, with a more conspicuous structure; the contours of the branches are more distinct; the triangular form of their cross-section is very pronounced, the sides being somewhat concave. On very narrow capillaries these changes are further accentuated. The protoplasm is packed about the nucleus and the branches

are short and stout, though still encircling the capillary and ending in sharp points.

When the branched cells are followed along a capillary toward an arteriole or a venule their shape gradually becomes different. They become shorter; the nuclei do not lie exactly in the direction of the capillary, but more or less slantingly around it; the branches are reduced in number and length along the capillary. On the arterioles themselves every stage of transition can be found between normal spindle-shaped muscle cells with an elongated nucleus, encircling the vessel, and others with an oblique nucleus and the protoplasm split up into a few ramifications. These latter become more numerous on approaching the capillaries, and there is no sharp distinction between them and the richly branched cells characteristic of the capillary.

By supravital staining with methylene blue the pericapillary cells take up the stain only at a certain stage, while later it disappears and is probably decomposed. The cells are not stained in their entirety, but the nuclei and certain fibrils in the protoplasmic branches take up the stain and deposit it as fine granules. From the analogous staining of the undoubted smooth muscle cells on arterioles it is concluded that the fibrils represent the contractile elements in the cells.

I reproduce three of Vimtrup's figures showing smooth muscle cells from an arteriole and the complete series of transitional cell forms connecting these with the typical branched cells on a capillary.

Fig. 24 shows the point of branching of an arteriole with circular, smooth muscle cells around the endothelial tube. A few of these cells are simple spindles, while the protoplasm of others is broken up into two or more parallel threads, and others again show a quite irregular ramification. The nuclei (*a*) of the more or less regular forms are arranged as arcs of a



circle at right angles to the direction of the vessel, while the nuclei of the others occupy a slanting position. The nuclei of the endothelial cells (*b*) are visible in the figure, but are not very distinct.

Fig. 25 shows a large capillary with typical branched cells in considerable number, though much fewer than on the arteriole. The threads, which run from the nuclei (*a*) round the circumference of the capillary, probably represent the muscle fibrils and not the entire

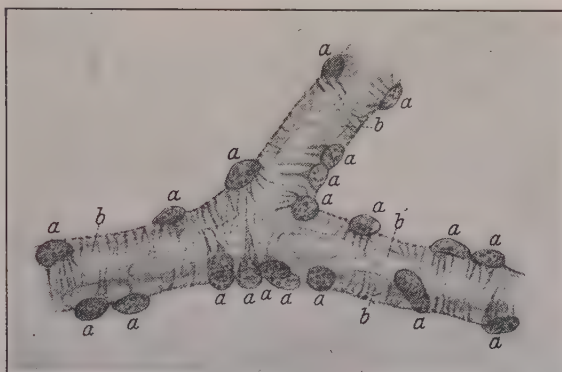


Fig. 24. Transition between arteriole and capillary.

protoplasm of these cells. The granular appearance of the threads has probably nothing to do with the structure of the fibrils, but is a simple deposition of methylene blue. In Fig. 26, which shows a small and slightly contracted capillary of  $8\mu$  diameter, the cells are fewer in number. Their nuclei are arranged lengthwise on the vessel, and in one place the elongation of the cell along the capillary wall can be distinctly seen. Red blood corpuscles, *r*, are also shown.

Vimtrup's methylene blue preparations have been examined by several very competent histologists

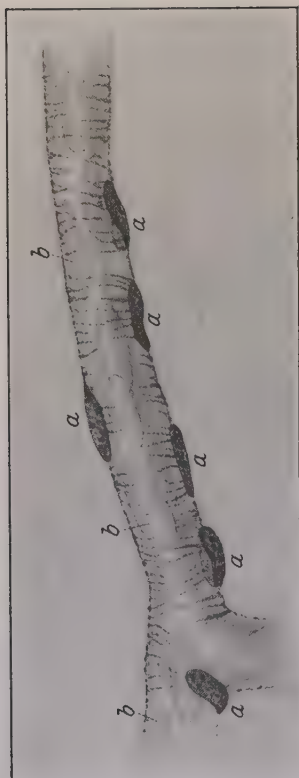


Fig. 25. Large capillary.

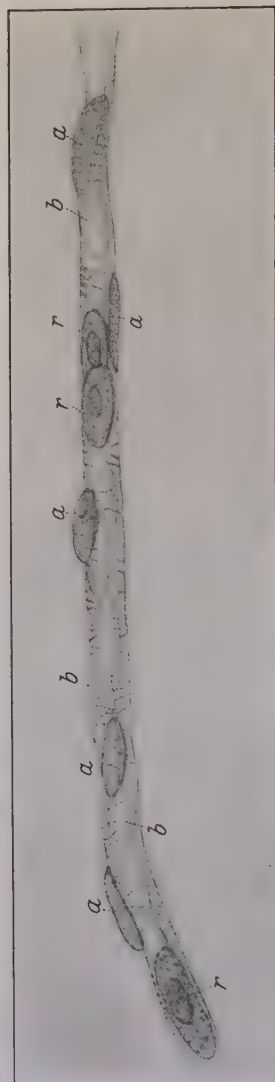


Fig. 26. Small, somewhat contracted capillary. *a* Muscle cells, *b* Nuclei of endothelial cells, *r* Red corpuscles. From nictitating membrane of frog. Supravital staining with methylene blue.  $\times 500$ . After Vimtrup.

(F. C. C. Hansen, Spalteholz, Bensley, and others) who have declared themselves entirely convinced regarding the muscular nature of the cells stained.

As there can be no doubt that the richly ramified muscle cells on the capillary wall are the same as those originally found by Rouget in the hyaloid membrane,

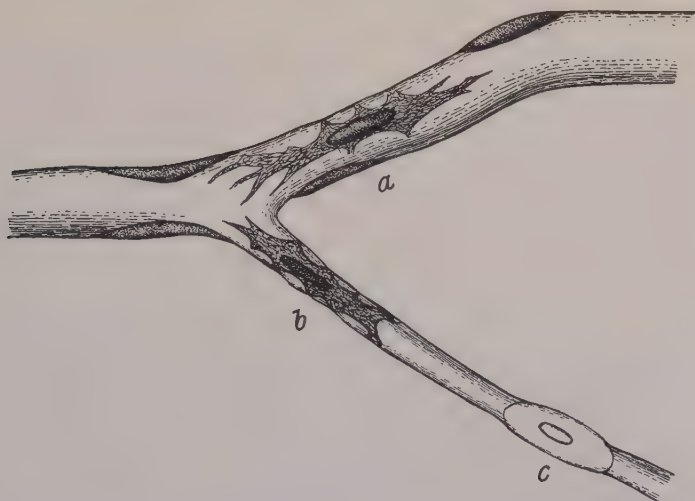


Fig. 27. Two Rouget cells (*a* and *b*) as seen on capillaries in living newt larvae. *b* is contracting, *c* is a red corpuscle.  
×500. After Vimtrup.

Vimtrup has named them after the first discoverer, and we shall speak of them, henceforward, as *Rouget cells*.

After having studied these cells in stained preparations so as to be completely familiar with their distribution and appearance, Vimtrup has further succeeded in observing them on living capillaries and has been able to follow in a single cell the changes of form taking place by contraction. The best object for these

observations he has found to be the tail of young newt larvae (*Triton punctatus*), which can be arranged for observation even with oil immersion lenses. By a simple and ingenious arrangement Vimtrup has been able to immobilize these larvae and keep them in excellent condition for hours without having recourse to narcosis.

In these animals spontaneous contractions and dilations of single capillaries or parts of capillaries are of frequent occurrence, and it is a significant fact that a contraction always begins just where the nucleus of one of the Rouget cells is located. During the process of contraction the cell shows the different forms described as characteristic in the case of stained preparations, but the movements themselves are generally too slow to be followed by the eye.

This can be done, however, when contractions are induced by stimulation of nerves in the web of a small frog. After a latent period of about fifteen seconds the Rouget cell under observation will show an increase in the refraction of light, and a few seconds later the contraction proper will begin. The nucleus of the cell is observed to sink a little into the capillary, and on the opposite wall several small indentations make their appearance. Some of the ramifications, as a rule, become distinctly visible, when the capillary is already somewhat contracted, and it can be observed that their positions correspond to the indentations seen in the endothelial wall. After two to three minutes' stimulation a maximum, though usually incomplete, contraction is generally obtained, but about this time a very curious change takes place in the tissues, which lose their normal transparency and become so opaque that no structural details can be observed. When the stimulation has ceased the tissues regain their normal transparency and the contracted Rouget cells relax in the course of a few minutes.

*The changes in the endothelium by contraction and dilatation.*

On special preparations Vimtrup has studied the appearance of the endothelial cells and their nuclei, corresponding to the different states of contraction and dilatation of capillaries. On dilated capillaries the edges of the rhomboidal endothelial cells are more or less straight, the cells themselves are large and their nuclei are thin, oval disks, which project only very slightly, if at all, on the inside of the capillary tube. On somewhat contracted capillaries the endothelial cells are smaller, their edges are sinuous, as shown in Fig. 23, their nuclei are more or less ovoid and project into the lumen of the capillary tube. On capillaries which are strongly contracted the endothelial wall becomes folded. That folding takes place was strongly maintained by Steinach and Kahn, and Vimtrup, too, has observed living capillaries, the appearance of which strongly suggests a folding of the wall, but he found it impossible to obtain decisive evidence on the point.

Examining the very object used by Steinach and Kahn, the nictitating membrane of young frogs, and stimulating the vessels to contraction by faradic currents, Rehberg has succeeded in my laboratory in demonstrating with absolute certainty the folding of the endothelium in strongly contracted capillaries. When a capillary is watched under a high power during the contraction, the formation of the folds can be very distinctly seen. Occasionally the folds protrude into red corpuscles which can be squeezed out into very strange forms. In the arterioles with their larger internal surface the folding of the endothelium by contraction is even more pronounced and very easy to observe.

Vimtrup's results have not been universally ac-



cepted and, although they can be shown to be substantially correct, it must be admitted that there are still a number of obscure points with regard to pericapillary cells and that some confusion is inevitable so long as no reliable methods have been elaborated by which the different kinds of such cells can be definitely distinguished.

In 1923 Zimmermann published his studies, continued for thirty-seven years on the structure of capillaries in a number of organs in man and representatives of all classes of vertebrates. In these researches Zimmermann has demonstrated by means of an impregnation method (Golgi-Kopsch) the universal existence of "pericytes." On the small arteries these are very similar to the well-known smooth muscle cells, on the arterioles they depart more and more from this form and assume finally on the capillaries the form of richly branched cells encircling the vessel with their twigs. On the venules the branches again become fewer and shorter, and finally we have again forms like ordinary plain muscle cells and Zimmermann himself is explicit regarding the muscular nature of the pericytes. Several of Zimmermann's pictures are very similar to Vimtrup's, but others are extremely complicated and scarcely correspond to histological realities as pointed out by Spalteholz (1927, p. 413). Others again perhaps correspond to pericapillary cells which are not Rouget cells, and I have to admit that Zimmermann's results do not inspire complete confidence.

That "adventitial" cells exist has been known for forty years. They are easily stained and take up intravital such colloidal dyes as pyrrhol- or trypan-blue, they may belong to the omnipresent, but evasive, reticulo-endothelial system of which so much is heard nowadays, but they have nothing to do with the Rouget

cells, which are very difficult to stain and which take up *intra vitam* only certain diffusible dyes which are *temporarily* deposited in their fibrils, the appearance of which when thus stained cannot be distinguished from the myofibrils of undoubted smooth muscle cells. When a series of authors (Marchand, 1923; Maximow, 1926; Aschoff, 1924; Ferrata, 1925; Benninghof, 1926; Ferrario, 1926; Volterra, 1925; and others) include the Rouget cells in one group with all other adventitial cells and deny their contractility on histological grounds they have failed to grasp this essential difference although it was emphasized by Vimtrup and Zimmermann.

Florey and Carleton (1926) have made intra-arterial injections first of formaldehyde to fix the vessels in the mesentery of cats and dogs and then of a staining solution (iron hematoxylin). They claim that this method gives the clearest pictures of the capillary wall obtainable. They describe and depict capillaries with a small number of nuclei, about 12 on a length of  $300\mu$ , which they take to be endothelial nuclei, while they have seen only single nuclei at points of branching which might belong to Rouget cells. Even around these they have failed to find any trace of a branching cytoplasm. While their methods could scarcely reveal the existence of cytoplasm in any case, the number of nuclei found by them is much too small to represent the endothelial cells. They may very well belong to Rouget cells, but nothing definite can be said.

The negative results of E. R. and E. L. Clark (1925, 1 and 2) are of more importance, though in my opinion not conclusive. These authors attempted to study the development of Rouget cells on sprouting capillaries of very young tadpoles. They have kept these under observation for a long time, making drawings of vessels and cells every day. They come to the conclusion

that cells, which they assume to be identical with Vimtrup's Rouget cells, are derived from connective tissue cells which come to associate themselves with the growing vessels and that these cells are devoid of contractility. According to Vimtrup the capillaries of very young larvae exhibit no contractility. There is no proof that the cells described by the Clarks are Rouget cells, and they have failed to make out the origin and multiplication of the smooth muscle cells of arterioles.

In their second paper (1925) Clark and Clark have studied the contraction of capillaries in tadpole tails and maintain that it is independent of the alleged Rouget cells. They describe two types of contraction. One is brought about by interference with the circulation as a whole and appears to be a simple passive retraction of the endothelial tube, probably due to the reduced pressure. The other type is brought about either by increasing the strength of chloretone, used as a narcotic, from 1 in 5,000 to 1 in 3,000 or by reducing its temperature, say from 30° to 26° C. In these contractions the outline of the capillaries becomes irregular and "wavy," but it is stated that the indentations have no special relation to the "Rouget" cells. They start more often at a distance from such nuclei and occur on quite young capillaries on which no adventitial cells could be made out. It is very unlikely that a comparatively slight increase in chloretone concentration or a slight reduction in temperature should induce contractions of Rouget cells, and the observations have probably nothing to do with the normal contractility here discussed, but they may be worth following up, because pointing to the possible existence of mechanisms for contraction or shrinkage in the endothelium itself.

Tannenberg (1925, 1) has made observations on the living mesentery of the rabbit which completely con-

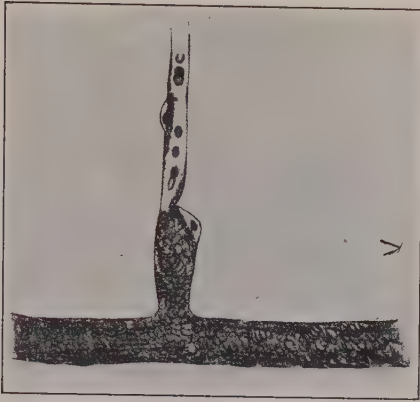


Fig. 29. Capillary with two Rouget cells of which one acts as a valve.  
After Tannenber.

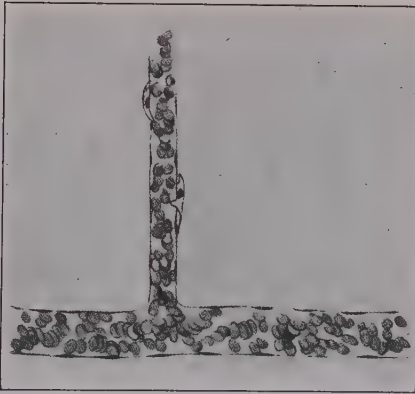


Fig. 28. Capillary with two Rouget cells.  
After Tannenber.

firm Vimtrup's results. His figures reproduced here (Figs. 28 and 29) show the existence of a large number of endothelial nuclei and in addition a small number of cells lying on the outside of the capillary wall which can be observed to contract and constrict the capillary, the wall of which may be seen to become folded. The cytoplasm cannot without staining be observed in its entirety, but Tannenberg has seen how the nucleus becomes more rounded and is surrounded by more cytoplasm, when the cell contracts, just as described by Vimtrup. Large Rouget cells, "Pfortnerzellen," appear to be in the mesentery normally arranged at or near the points of branching of capillaries.

Very careful observations on human nail fold capillaries have been made by Heimberger (1925) showing that after stimulation of a single point in the wall of a capillary or venule a constriction, usually very considerable, will take place in the column of blood, while a slow flow of corpuscles may be confined to narrow channels near the circumference of the vessel as well illustrated in Fig. 30. Heimberger shows by a detailed analysis that the only possible explanation is a longitudinal folding of the capillary wall under the action of a contractile mechanism situated on the outside.

In several cases and notably after the application of adrenaline Heimberger finds a concentric constriction of the column of blood which he is inclined, as far as I understand, to ascribe to a contraction of the endothelium itself. While not denying the possibility that endothelial cells may show contractility I cannot accept the present evidence for ascribing such a power to them, and especially in the case of adrenaline the essential similarity of the Rouget cells with the smooth muscle cells of arterioles would point to these as the elements brought to contraction by adrenaline.

In a dissertation published in the Dutch language



by Schaly (1926) the Rouget cells have been demonstrated on a number of capillaries in the human eye. Schaly has worked on fixed tissues and stained with histological stains according to Delafield, Heidenhain, Callaja, and others. He has worked out his methods on frogs' tissues and confirms Vimtrup with regard to



Fig. 30. Successive pictures of the blood column in a capillary loop after stimulation, showing that the wall must become folded to produce narrow channels traversed by single corpuscles. After Heimberger.

the existence and general appearance of the Rouget cells in the tongue of the frog (Fig. 31), the hyaloid membrane, and the tail of tadpoles. He emphasizes the general occurrence of strong Rouget cells at points of branching both of capillaries from arterioles and within the capillary network proper.

In the human eye he has studied the retina, choroid, ciliary body, iris, conjunctiva, Horner's muscle, orbital fat, and an abscess in the corpus vitreum with newly

formed blood vessels (Fig. 32). Rouget cells have been found in all cases, and as in the frog they are almost always present at points of branching. Usually only the nuclei are definitely stained, but in many instances,

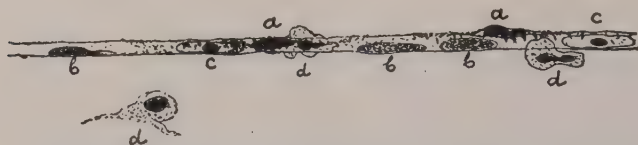


Fig. 31. Narrow capillary in frog's tongue, *a* Rouget cells, *b* endothelial nuclei, *c* red corpuscles, *d* white corpuscles.  
After Schaly.



Fig. 32. Newly formed capillaries in the corpus vitreum of the human eye. After Schaly.

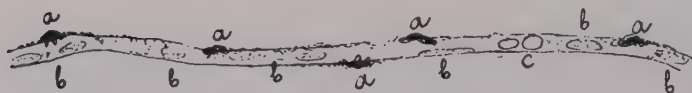


Fig. 33. Iris capillary with pigmented Rouget cells.  
After Schaly.

part at least of the protoplasm has been observed. This is the case especially in the iris where the Rouget cells and the muscle cells of the dilator pupillae contain a natural pigment (Fig. 33).

Fig. 34 shows a small artery and vein from the choroïd membrane with the intervening capillaries in

which the transition from the normal smooth muscle cells on the large vessels to the Rouget cells is clearly exhibited. Schaly gives many measurements of the distances between Rouget nuclei. Behind the macula he finds an average distance of only  $30\mu$ , in the equatorial part of the choroid the distance is  $56\mu$ , and in the periphery  $74\mu$ .

Very recently Bensley and Vimtrup (1928) have repeated and extended Vimtrup's observations on living

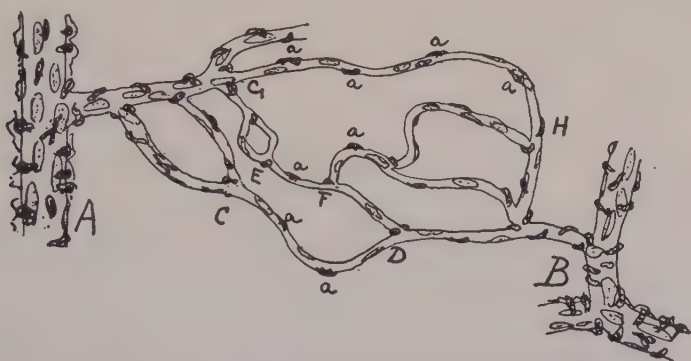


Fig. 34. Transition from an arteriole (*a*) in the choroid to a venule. After Schaly.

capillaries using the excised nictitating membrane of frogs in which contractions of all vessels are easily produced by faradic stimulation. They have distinctly observed the action of the pericapillary cells and the longitudinal folding of the endothelium which can be best seen on venules owing to their larger caliber. This object can be recommended to any skilled microscopist who wishes to convince himself of the reality of the activities of Rouget cells.

Bensley and Vimtrup have also confirmed the results obtained with methylene blue used supravitaly, and by utilizing Janus green as a special vital stain for

myofibrils they have been able to show that the normal smooth muscle cells of arteries in the nictitating membrane contain bundles of fibrils running in an almost circular course round the vessel. In the more scattered cells on arterioles the fibrils belonging to one cell often form two or three distinct groups and on the true capillaries the quite similar fibrils of one cell are spread out into several small groups, each of which encircles the vessel with its tapering ends.<sup>1</sup>

An attempt to combine all the observations into a coherent conception of the anatomy of a "normal" capillary will give about the following result: Like the walls of the smallest arteries and veins, the capillary wall consists of two distinct elements—the endothelial tube and the outside muscular coat. The important difference between capillaries and larger vessels lies in the arrangement of the muscles, which in the arteries and veins form a more or less continuous layer, greatly increasing the thickness of the wall and offering a considerable resistance against the exchange of substances between the blood and the surrounding lymph spaces or tissue cells, while in the capillaries the muscular coat is arranged more or less in the form of a wide-meshed network, leaving the larger part of the endothelial surface uncovered and adapted for the passage of substances with a minimum of resistance.

The muscle cells contain fine fibrils which constitute the contractile element proper, while the cytoplasm and the nuclei are passively stretched and flattened out when the fibrils relax.

The muscular coat of the capillaries possesses, like smooth muscles generally, a definite tonus, or "posture," in the terminology of Sherrington (1920), subject in the organism to nervous, hormonal, and other influences, and the diameter of a capillary is, on the whole, determined by the state of contraction of its

muscular coat. The changes observed in the configuration of the endothelial cells and in the shape of their nuclei make it clear, however, that the endothelial tube itself must possess some normal form and caliber which it will assume when the outside and inside pressure is absolutely the same. When the outside pressure is the higher, the tube collapses. When the Rouget cells contract sufficiently, it becomes folded, and when they relax and the inside pressure is ever so slightly higher than the outside, it is dilated, and the endothelial cells are passively stretched, their surface is enlarged, their thickness is diminished, and their nuclei are flattened out.<sup>2</sup>

The force necessary to stretch the endothelium—apart from the muscular coat—must be extremely slight, since the capillaries can, as I have shown, be opened up by a minimum of pressure, when their muscles are relaxed.

There is, so far as I can see, no definite evidence that the endothelium itself is contractile. It must possess as I have said some normal form or posture, and it seems very probable that this form and the force with which it is maintained is subject to changes. The normal endothelium must be able, even when considerably expanded, to resist the blood pressure exerted upon it in the free spaces between the Rouget fibrils. In the first of these lectures I mentioned the varicose appearance sometimes, though by no means regularly, shown by dilated capillaries. This may be due to the endothelium giving way between the lines of the Rouget fibrils.

The descriptions given above of the endothelium and Rouget cells apply to the capillaries of amphibia generally, where the contractile elements have been observed on so many different capillaries that they can safely be taken to exist in almost all tissues. They have been seen by Rouget on the capillaries in the hyaloid



membrane of the frog's eye and on those in the tails of tadpoles and newt larvae; by Mayer on smooth muscle capillaries in the wall of the intestine and the urinary bladder of frogs and newts, and Vimtrup has found them further in cutaneous capillaries, in those of the mucous membrane and striped muscle of the tongue and the nictitating membrane of frogs. As a rule, especially large and powerful Rouget cells are found at or near the points of branching of capillaries.

The Rouget cells appear to differ considerably in shape and structure, according to the organ where they are found. In the tail of amphibian larvae and in the web of grown frogs they are of a rather primitive ameboid character, with much undifferentiated protoplasm and few muscle fibrils. Their total length varies in the larval capillaries from about 60 to 200 $\mu$ , and in the frog's web from 40 to 80 $\mu$ . The number of cells per mm. of the capillary in the web has been found in a single count to be about 70.

In the tongue and nictitating membrane the cells are more robust and distinctly muscular, with a large number of fibrils. Their total length varies between 40 and 80 $\mu$ , and from 20 to 50 have been counted per mm.

In the hyaloid membrane the cells are extremely elongated along the capillary, giving off in a very regular manner hooplike branches encircling the capillary tube.

The normal thickness of the endothelium in a capillary which is neither dilated nor contracted appears to be somewhat less than 1 $\mu$  (about 0.8 $\mu$  probably). On a dilated capillary the thickness is reduced by stretching in proportion to the increase in diameter.

In mammals the Rouget cells have been described by Rouget himself in the retina and fatty tissue in rabbits and in the brain of ruminants. Vimtrup has found them in interstitial connective tissue in man and in the

intestine of mice and it should be mentioned that as early as 1920 the late Professor E. Müller of Stockholm showed me their nuclei on capillaries in the intestine of the cat while Tannenberg has later observed their contractions in the mesentery of the rabbit. That they occur regularly on the cutaneous capillaries and venules of man has been shown by Vimtrup and Heimberger (see p. 98 and Fig. 35), while Schaly has demonstrated their existence in practically all tissues of the human eye. If we accept the evidence given by Zimmermann they exist in practically all tissues of mammals and also in birds, reptiles, and fishes.

*The development of capillaries.*

In the vertebrate embryo the vascular system begins as "angioblasts," which are very early differentiated from other mesenchymal cells. When the angioblasts divide they form syncytial masses which possess two essential properties, viz., "(1) the power of liquefying in the center, with the formation of plasma and vesicles (2) the power of sprouting, by which these groups of cells join similar groups, forming vessels or plexuses." (Sabin, 1920, 1922.)

According to this conception which is, I understand, now generally accepted, the vessels begin as endothelial tubes to which adventitial cells are later associated. The power to form new angioblasts appears to become lost early in embryonic life. In the chick embryo the differentiation of angioblasts is extremely extensive during the whole of the second day, from the third day on it becomes relatively greatly diminished, but it has been followed by Miss Sabin up to the seventh day. All later vessels are formed by sprouting from the preëxisting system.

In the process of sprouting single endothelial cells start as outgrowths from capillaries or somewhat

larger vessels, and it appears that a lumen can be formed very early inside such single cells. As mentioned above E. R. and E. L. Clark have seen connective tissue cells (nuclei) join the growing sprouts as adventitial cells. Miss Sabin describes and depicts sprouting veins from the embryo chick in which adventitial nuclei follow closely in the train of the growing endothelium from the parent vein.

It should be pointed out that the origin of the muscle cells whether on capillaries or larger vessels has not been made out. It seems probable that in a growing vessel these are derived from the muscle cells of the parent vessels, while other adventitial cells join from the surroundings.

*The specialized blood vessels of certain organs.*

In a number of animals throughout the vertebrate series we find in certain organs highly specialized vascular structures. It is impossible in this brief account to deal with these, and I shall here only describe the main features regarding the structure of capillaries in the liver, in the glomeruli of the kidney, and in the human skin.

The endothelium of the hepatic capillaries appears to be a syncytium with numerous nuclei, but without cell borders as in embryonic capillaries. At short, rather regular, intervals, the star-shaped cells of v. Kupffer (1876, 1899), the function of which has been shown to be phagocytic, form an integral part of the capillary wall. A further very remarkable feature in the structure of the hepatic capillaries are the "canaliculi" inside the liver cells, which communicate directly, according to the researches of several authors (Brovitz, 1899; Schafer, 1902; Herring and Simpson, 1906), with the lumen of the capillaries. According to v. Kupffer the liver capillaries are surrounded by an

adventitial layer, perhaps constituting a pericapillary lymph space. Rouget cells have not been observed.

It is easily observed and has been known for a long time that the liver will take up at an almost incredible rate microscopic particles of any kind, including living and dead bacteria (Rosenthal, 1921; Oerskov, 1925) and it has been shown that the function is subserved by the Kupffer cells which, according to Pfuhl (1926), take up normally a definite capturing attitude ("Fangstellung") with a meshwork of pseudopodia projecting into the lumen of the capillaries. These threads are sticky and become visible by a coating of carbon particles, when Indian ink is injected vitally into the circulation. Somewhat later the particles are taken up by the pseudopodia and in the position of digestion these are retracted and drawn into the capillary wall. When a suspension of Indian ink containing milliards of particles per cubic mm. is slowly injected into the portal vein of a frog only a small fraction of these will, up to a certain point, pass through the liver and appear in the general circulation. The bulk are retained by the Kupffer cells.

The power of phagocytosis and its concomitants, the property of stickiness, and power of ameboid movement, are not confined to the Kupffer cells, but appear to be developed to some extent in the vascular endothelium generally (Rosenthal, 1921; Herzog, 1925; Sabin and Doan, 1926; Stilwell, 1926). This is strongly emphasized by Ebbecke (1923), who ascribes to the ameboid character of the endothelial cells a large part of the capillary contractility. While I am unable to follow Ebbecke in ascribing on the evidence at present available any significant part of the general contractility of capillaries to the endothelial cells, I have to admit that they possess a definite power of ameboid

movement manifest especially in the process of sprouting referred to above.

The capillaries of the liver are normally much more permeable to dissolved substances of a colloidal nature than those of other organs (Lecture XIII). This peculiarity may be correlated with the existence of the "canaliculi," but the inference is by no means binding since any capillary will, in certain conditions to be discussed later, acquire the same permeability.

With regard to the glomerular capillaries in the mammalian kidney it was until recently generally assumed by histologists and physiologists that the glomerulus was formed by a close-meshed network of anastomosing capillaries and surrounded by the visceral layer of capsular epithelium as diagrammatically depicted by Cushny (1917, second edition, 1926). Certain histologists (Schaffer, 1920) have observed that the capsular epithelium penetrates deeply into the glomerulus and divides it into a small number of distinct lobes, but Vimtrup (1926) has shown that the glomerulus is built up of a large number of distinct capillary loops each running without a single anastomosis from the afferent to the efferent artery and each covered with a layer of capsular epithelium the nuclei of which are very conspicuous. Endothelial nuclei have not been observed and Rouget cells appear to be absent. Vimtrup's result has quite recently been confirmed by v. Möllendorf (1927) who uses the term "Deckzellen" to denote the layer of capsular epithelium.

In the frogs' glomeruli Richards and Schmidt (1925) have found evidence by direct observation of the living kidney of contractile elements situated at the points where the glomerular capillaries branch off from the afferent artery.

In all the capillary fields which have been closely ex-



amined there is a very distinct difference between arterioles and capillaries. The arterioles have a well-developed muscular coat of simple circular fibers, covering the entire surface of the endothelial tube. Proceeding from an arteriole to a capillary there is a *short* zone of transition, as shown in Fig. 24, in which the number of muscle cells is reduced and the endothelium uncovered, but, on the whole, there is a sharp demarcation between the muscular arteriole and the capillary, the distinctive feature of which is the large extent of free endothelial surface in proportion to the area covered by the contractile elements.

Until recently the human skin was supposed to provide an exception from this general rule, since Spalteholz (1893) described the arteries and arterioles in the outer half of the corium as being devoid of a muscular coat. Vimtrup (1923) was able to demonstrate in preparations from the cutis that the muscularis was continued as a single layer of spindle cells. Below the subpapillary net the layer becomes incomplete and in this net and its branches we have endothelial arterioles with single muscle cells which occupy a more and more slanting position as we approach the capillaries proper. On the capillaries and venules Vimtrup observed nuclei which he was able to identify as belonging to Rouget cells (Fig. 35). Although the branching protoplasm has not been observed the two facts (1) that these nuclei clearly belong to the same category as the undoubted muscle nuclei on the arterioles and (2) the observations by Heimberger (1925, 1) of a contraction of human skin capillaries and venules bringing about a longitudinal folding of the endothelium show conclusively that his interpretation is correct. Withdrawing his earlier statement Spalteholz (1927) has confirmed and amplified Vimtrup's result concerning the muscular coating of the outer arte-

rioles, but he declares himself unable to distinguish Vimtrup's Rouget nuclei on the capillaries from other adventitial nuclei. He finds as a rule a continuous or

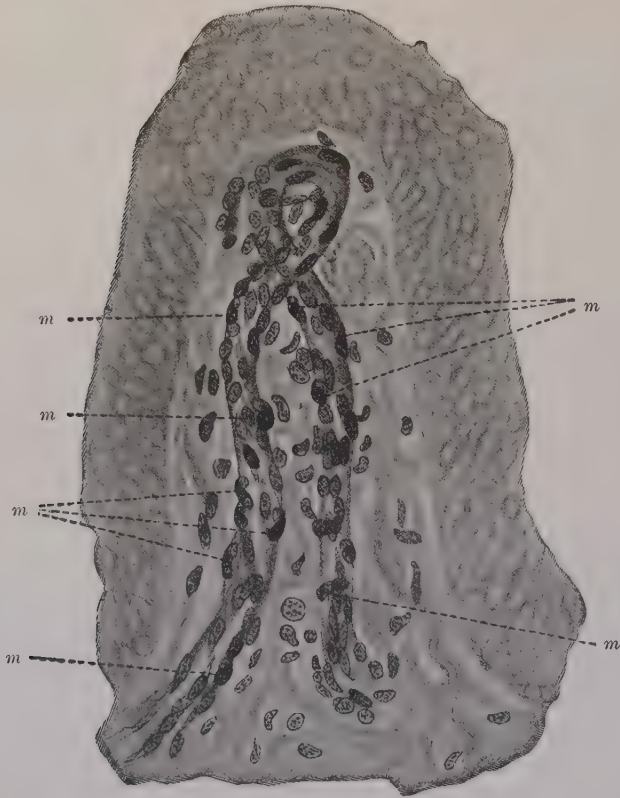


Fig. 35. Capillary loop from human skin, *m* nuclei of the Rouget cells,  $\times 500$ . After Vimtrup.

almost continuous layer of muscle cells even beyond the subpapillary arterial plexus and sometimes right up to the capillary loops. There can be no doubt, therefore, that the arterioles of the human skin conform to

the general rule, but it should be pointed out expressly that it is by no means impossible that vessels showing single muscle cells arranged at intervals and with the nuclei more or less in the direction of the vessel can function mainly as arterioles supplying a net of functional capillaries. It is required only that the Rouget cells are strong enough and possess a sufficient tonus to keep them so narrow that the velocity of flow in them is much higher than in the capillaries proper.

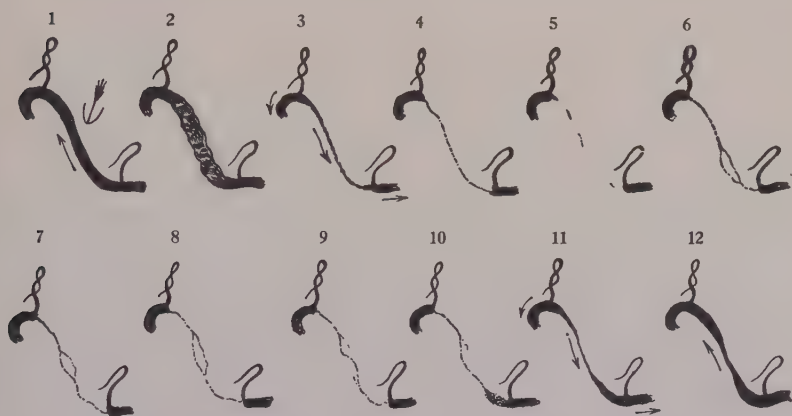


Fig. 36. Subpapillary vein after mechanical stimulation. Folding of wall shown by flow of corpuscles in variable paths.  
After Heimberger.

Heimberger (III, 1926; V, 1927) has made the interesting observation on the nail fold capillaries of man that the capillary loop in each papilla is surrounded by a continuous lymph space traversed by a number of threads or septa, which keep the loop in position. When a fine needle cathode is introduced into this space it becomes gradually filled with hydrogen bubbles and when a colloidal dye, like trypan blue, is

injected the whole space is filled, and the dye solution penetrates between the cells of the surrounding tissue. It cannot be doubted that these lymph spaces are continuous throughout the corium, and the significance of this will come up for discussion in a later lecture.

It can be stated generally that the difference between capillaries and venules is not pronounced either histologically or physiologically. The muscular coat of many venules is so thin and incomplete that the exchange of substances through their walls must be considerable, and in some cases the vessels, which are anatomically classed as veins, are from a physiological point of view to be regarded as capillaries, since, in spite of their comparatively large size, they have a coating of Rouget cells instead of simple muscle fibers, while the greater part of their surface is uncovered. The subpapillary veins of the human skin are in reality such "*giant-capillaries*." Spalteholz (1893, 1927) describes them as having no muscular coat, but Vimtrup (1923) has succeeded in observing on stained preparations the nuclei of their Rouget cells. In their physiological reactions to stimuli they behave just like capillaries as will be shown in later lectures and Heimberger (1925, 3) has observed the folding of the walls after stimulation of a single point, just as in capillaries.

*The direct communications between arteries and veins and their significance.*

In the anatomical literature of fifty years ago there are a number of references to the existence of "*derivating channels*," represented by small arteries opening directly into somewhat larger veins. Such connections have generally been observed on injected specimens and without verification by a study of the

structure of the vessels in question. As single capillaries can easily become greatly dilated by injection under pressure and give the appearance of wide channels, evidence of this kind is obviously untrustworthy.

Hoyer (1877) describes the existence of direct anastomoses between arteries, generally of about 0.02 mm. diameter, and slightly larger veins as occurring only in certain places in the body of mammals. He has found them near the edge of the ears in rabbits, dogs, and cats, in the tip of the snout in several animals, as well as in the tip of the tail, and finally in the tips of fingers and toes in man and other mammals. In many of his cases he has studied the structure of the vessel wall in the places of anastomosis after suitable staining, and he describes and pictures vessels of undoubted arterial structure opening directly into others which must be accepted as veins. He has made a search for these "derivating channels" in many other places, but apart from the well-known case of the arteries opening directly into the corpora cavernosa, he has found them only in such projecting parts as might require a considerable supply of blood to keep them warm, when exposed to low temperatures.<sup>3</sup>

Grosser (1902) confirms Hoyer's results as to the existence of derivating channels in the skin of the extremities and gives a careful description of their structure. They are numerous in the skin of human fingers where they are arranged in small groups 1-2 mm. apart. Their musculature is twice to thrice as strong as that of arteries of the same bore (about 0.02 mm.) and they are imbedded in connective tissue with numerous nuclei. They are common also within the digital bones, where they are surrounded by a venous plexus which allows them to open and close freely. They are especially large (0.1-0.2 mm.) in the thumb of bats.



Grosser has made a search for them in appropriate places in reptiles, where they appear to be absent.

In the last few years evidence has been brought forward for the existence of arterio-venous anastomoses from *intra vitam* observations of the circulation in the human skin. Heimberger (1925, 1) has been able to observe such connections directly and in considerable numbers in the fingers of persons having a very delicate skin. He describes them as short connections between peripheral arterioles and venules, short-circuiting the long capillary loops, and gives a number of figures of which I reproduce one (Fig. 37). He finds these channels normally closed, to be opened up for a short period by weak mechanical stimulation.



Fig. 37. Arterio-venous anastomoses. After Heimberger.

I should not attach much weight to these observations, which must be exceedingly difficult to verify, but for the two facts that Heimberger is an observer of quite extraordinary ability and patience, and that his direct findings are supported by observations of the blood flow in superficial venules and capillaries which are explicable only if arterio-venous anastomoses exist

in close proximity to the vessels under observation. In a few cases Heimberger (1925, 2) has seen pulsation of the blood in venules when the corresponding arterioles were closed and the blood in the capillaries quiescent. This is possible only when the venule in question possesses another connection with the arterial system, through which the pulse is admitted. In other cases the blood is seen for some time to flow back from a venule through the capillaries to an arteriole, which postulates that the venule is connected directly with a somewhat larger artery, transmitting sufficient pressure to carry the blood back into an arteriole which must also be in direct communication with a larger vein.

It follows from the observations of Heimberger that his "derivating channels" which are distal to those of Hoyer and Grosser must be able to contract and expand and to close up entirely.

It must be borne in mind that the arterio-venous anastomoses here dealt with have nothing in common with the pathologic arterio-venous fistulas of the gross anatomy. Though wider than capillaries, when fully dilated, they are much too small to be seen with the naked eyes and cannot in any way imperil the circulation even when present in very large numbers. The blood passing through them will probably fail to give off the normal amount of oxygen and other substances which it is supposed to carry to the tissues, because it is not exposed to the thin walls and large surface of the capillaries, but for the dissipation of heat the surface available in vessels of less than  $1/10$  mm. diameter is ample.

It seems unnecessary to show this by an elaborate calculation of the rate at which heat can be given off from the surfaces of capillary tubes, when it is remembered that according to the calculation first made by

Stewart (1895) 1 cubic millimeter of blood takes over 6 hours to pass a capillary of  $10\mu$  diameter when flowing at the high rate of 0.5 mm. per sec. For the equalization of the temperature of 1 mm.<sup>3</sup> of blood with the surrounding tissue 1 second is probably more than



Fig. 38. Blood vessels of *Nereis*.  
After Retzius.

sufficient, and this would be provided in a vessel of  $100\mu$  diameter, when the rate was 12 mm. per sec. That is why small arterio-venous anastomoses must be considered extremely efficient for keeping up the temperature of exposed parts, and I cannot doubt, considering the location of derivating channels described by Hoyer, that the main function of these vessels is to supply

enough blood to projecting parts of warm-blooded animals to keep them warm when exposed to low temperatures.

It would probably be worth while to make a search for such anastomoses in the ears, feet, and snouts of arctic mammals and in the feet of certain birds. When the penguins brooding on the South Polar Continent at temperatures far below  $0^{\circ}$  keep their one egg raised upon their own feet, there must certainly be a rush of blood through the web of those feet, and the existence of large and numerous arterio-venous anastomoses would seem extremely likely.

### NOTES

<sup>1</sup> *Rouget cells in worms.* It is, I think, very suggestive that cells absolutely similar to the smooth muscle and Rouget cells of vertebrates were described over 20 years ago on the blood vessels of annelid worms (*Nereis* and other forms) and have recently been shown experimentally to be contractile elements. Retzius observed these cells in 1891 and studied them in detail by vital staining with methylene blue in 1905. Three of his numerous figures are here reproduced. The cells stain along with and in the same way as undoubted muscle cells (just as Vimtrup's Rouget cells) and the great histologist says: "According to my view—and I have really now considerable experience in these matters—the cells in question on the blood vessels can scarcely be anything but muscle cells."

Federighi has recently (1927) studied the contraction processes in the living blood vessels of *Nereis*. He states that there are two types of contraction; one is peristaltic in character, it is independent of the central nervous system and is brought about by the endothelium, the other can be induced locally by stimulation and is brought about by Retzius' muscle cells. The first type will probably repay a closer study, it remains to be seen whether it has any analogy in the capillaries of vertebrates, or whether as a more primitive type it is confined to invertebrates. A regular peristaltic movement of the endothelium certainly does not exist in vertebrate blood capillaries though peristalsis has been observed in the lymphatics (Lieben, 1911; Florey, 1927).

<sup>2</sup> *Stretching and folding of the pulmonary epithelium.* We have in the stretching and folding of the capillary endothelium a close analogy to the behavior of the alveolar epithelium in the lungs of man, according to Marie Krogh's (1915) determinations of the pulmonary diffusion con-

stant. These determinations of the quantity of carbon monoxide which will diffuse into the blood from the alveoli, show that when the lungs are expanded beyond a certain point the epithelial surface becomes larger and thinner by stretching, but when they are allowed to collapse below that point the surface area and thickness remain constant—the surface is folded.

<sup>3</sup>In a very recent publication Wearn (1928, 2) demonstrates the existence of anastomoses in the heart of man and mammals between the coronary artery and the Thebesian veins. These latter vessels normally drain off a considerable proportion of the venous blood from the heart muscles directly into the right and left ventricles. The anastomoses with the artery are opened up when the heart, which has ceased beating, is dilated by injection. Their function during normal life is entirely unknown, but Wearn mentions two human cases where it was found at autopsy that the orifice of the coronary artery was entirely occluded and the heart muscle could receive its blood supply only through these anastomoses. The occlusion had developed slowly, but was of long standing, and the supply through the Thebesian anastomoses had enabled the patients to lead a normal working life.



## LECTURE V

### THE INNERVATION OF CAPILLARIES

THERE is scarcely any field in which close co-operation between anatomical study, physiological experimentation, and clinical observation is more urgently needed and promises to be more fruitful than in the investigation of innervation problems generally and that of the innervation of the small blood vessels especially. Microscopical anatomy alone can disclose the existence of the fine nerve fibers with which we have to deal. Experiments and observations are necessary to ascertain their functions and connections with the central nervous system. At present the clinical and experimental "results" are far advanced from their anatomical base, and Ph. Stöhr, Jr., (1927) may be right in contending that the advance has been too rapid, that we are in imminent danger of being cut off from our base, that our conceptions regarding the sympathetic system especially cannot be brought in harmony with its histological structure.

In this lecture we shall deal mainly with physiological and clinical results and it should be kept constantly in mind, even where it is not expressly stated, that these are to be regarded as nothing more than working hypotheses, unless and until they are confirmed anatomically.

#### *Sympathetic innervation of capillaries.*

When we accept the existence of Rouget cells constituting a muscular coat essentially analogous to that

of larger blood vessels, we must expect these muscles to be innervated through the sympathetic system.

Anatomically it has been shown long ago (Beale, 1860) and repeatedly confirmed on numerous tissues in different animals (Glaser, 1920), that the capillaries are regularly accompanied by nerves. Generally there are two fine, non-medullated fibers, running along each capillary, and connected by a number of anastomoses, crossing the capillary at angles of about  $45^\circ$  (Krimke, 1884). The nerves show small swellings at irregular intervals, but ganglion cells have not been found. The connections with the capillary wall were not observed until quite recently. Krimke's assertion that each capillary has its own nerves, which do not anastomose with those of other capillaries is certainly erroneous, and the spiral fibers observed on certain portions of capillaries (Glaser and others) are probably artefacts or muscles (Stöhr, 1926, Busch).

In recent publications it has been doubted and even positively denied that these nerve fibers can be responsible for the innervation of Rouget cells, since many of the cells and indeed many of the finest capillaries are without a nervous investment. I would point out in reply that even the finest histological methods now in use are probably unequal to the task of showing in their entirety the very finest nerve fibrils which we must expect to supply the single cells. The pictures, for instance, which Stöhr (1926) has obtained from the human heart by impregnation according to Schultze's silver method are certainly incomplete, in so far as a large number of fibers must have escaped impregnation, and misleading, in so far as many impregnated fibers appear to be much more robust than is at all likely.

Nordmann (1925) and Woollard (1926) who have stained the nerves of mammalian tissues vitally with

methylene blue give a description of the capillary innervation which does not differ from that generally accepted. Woollard has seen a rather diffuse plexus from which individual branches can be traced to the capillaries: "They can be traced for relatively enormous distances. As they pass further along the capil-

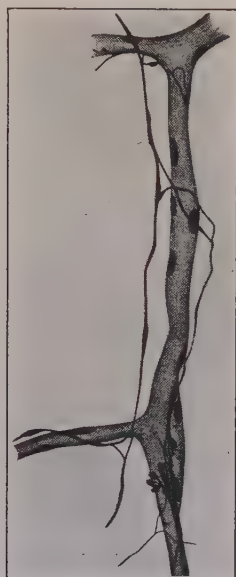


Fig. 39. Capillary nerves  
from human brain.  
After Stöhr, Jr.

laries they grow finer and finer and, without forming any sort of end apparatus, they pass beyond the range of visibility."

Dr. Busch who is at present studying vascular innervation problems here in Copenhagen by means of the methylene blue method (Rongalit white) will subscribe to the main part of the description given by Woollard,

but he finds in all cases a closed net of fibers along and between capillaries, and in a number of preparations, notably from the frog's web, he has succeeded in staining very fine fibrils penetrating into the Rouget cells. Fig. 40 which he has kindly placed at my disposal will give an idea of their appearance. Even in this case the staining is probably incomplete in so far as many of the finest fibrils remain invisible.



Fig. 40. Capillary nerves from frog's tongue. After Busch.

In the first edition of this book it was pointed out that the physiological facts pointed strongly to the existence of true peripheral nerve nets, in which conduction must be possible over fairly long distances in any direction. It is gratifying to find that the histological investigations have fully confirmed this concep-

tion in so far as the sympathetic innervation is concerned.

Woollard has shown that the nerve net supplying the capillaries in the mammals studied by him is in direct connection with the adventitial plexus and network of the arteries, and Busch confirms this for man and other mammals and also for the frog. They are both positive with regard to the essential point that while the nerves in the adventitia of larger arteries may form a plexus of fibers which do not really anastomose, the branches entering the muscular coat form a veritable network of freely anastomosing fibrils (Fig. 41). Woollard shows that on the large arteries the net is derived from non-medullated post-ganglionic fibers entering the aorta from the sympathetic ganglia, while farther out in the limbs this innervation is supplemented and replaced by non-medullated fibers entering the vessels from the peripheral nerves. The whole net is continuous, however, and degenerates when the sympathetic ganglia are excised, as already shown by Eugling (1908). The peripheral part does not degenerate in mammals after the so-called periarterial sympathectomy, and according to Busch it cannot be maintained by connection with sympathetic ganglia through the periarterial plexus alone when the spinal nerves are divided.

Physiologically it has been shown in a number of cases that stimulation of the dorsal sympathetic affects capillaries and causes them to contract with the arteries. As you will remember, this was found by Stejnach and Kahn on the frog's nictitating membrane, and their experiments were, in fact, guided by these considerations. In recent years the capillaries of several other tissues have been shown to be innervated through sympathetic fibers. Hooker (1920) found that electrical stimulation of the cervical sympathetic



brought about a very pronounced and independent constriction of capillaries and venules, as well as of arteries, in the skin of the cat's ear, and in my laboratory we have made the same observation on the ear of the albino rabbit, where very accurate observations can be made by transmitted light. An independent demonstration of the sympathetic innervation of the capillaries in the rabbit's ear was made recently by Harris and

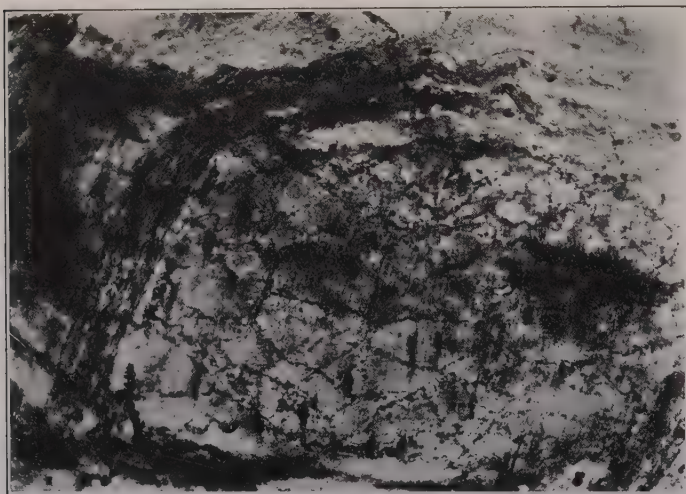


Fig. 41. Nerve net from arterial wall. After Busch.

Marvin (1927) who stimulated and saw the capillaries contract after obstructing the arterial flow completely. Leriche and Policard (1920) report a contraction of the capillaries at the base of the nail in man, when the sympathetic fibers running in the adventitial coat of the humeral artery are mechanically stimulated. The reaction is said to be practically instantaneous. On the hind legs of frogs we have made a more detailed study of the sympathetic innervation (Krogh, Harrop, and

Rehberg, 1922). We have stimulated the lower ganglia (8 to 10) of the sympathetic chain, which brings about a constriction first of the arteries and a few seconds later also of the capillaries of the web, as well shown on our circulation film. Generally we have not succeeded in making the capillaries contract to obliteration. As mentioned in the preceding lecture, with sufficiently high magnifications the contractions can be observed to start from those points where the nuclei of the Rouget cells are situated. The capillaries in the muscles of the leg are also influenced by stimulation of the sympathetic ganglia and sometimes contract completely. There is reason to believe that in this case every single Rouget cell is supplied from a sympathetic fiber and can be made to contract through it. Wernöe (1925) has obtained evidence to be mentioned later (p. 147), that the smallest blood vessels in the skin and intestines of fishes are under sympathetic innervation.

Hartman, Evans, Malachowski, and Michalek (1928) have recently observed that sympathetic stimulation causes *dilatation* of capillaries in mammalian muscles.

#### *Sympathetic tonus of capillaries.*

When the sympathetic ganglia are removed in the frog or the sciatic cut below them, the capillaries of the web dilate more or less permanently, from which fact it follows that they are, like the arteries, tonically innervated through the sympathetic system. Sometimes the dilatation takes place at once, as is the rule for the arteries, but more often it takes half an hour or more to develop. This might be supposed to be due to the stimulus of the nerve section, but mechanical stimulation has usually only a slight effect on sympathetic fibers and would wear off at any rate in a short time. Experiments by Drinker (1927) to be discussed

in detail in a later lecture show that even when there is no immediate dilatation the state of the web capillaries is profoundly altered very shortly after the nerve has been divided.

After section of the sciatic the tonus of the web capillaries is restored usually in a day, but after extirpation of the sympathetic ganglia they are, as a rule, very slow to regain their normal state, and in one case even remained dilated for a period of 100 days. This seems to indicate that tonic influences reach them via the arterial plexus.

Gabbe (1926) made vital injection experiments to show the sympathetic innervation of muscle capillaries. In frogs he divided the sympathetic nerves to one side and found after injection of Indian ink that the muscles on this side became more intensely blackened than on the control side while the microscope revealed an increased number and dilatation of capillaries. That the opening up and dilatation was not brought about passively by the increased pressure resulting from dilatation of arterioles, was shown by the fact that closure of the aorta, which reduced the pressure to a low level, did not prevent the dilatation. Similar results were obtained also on guinea pig muscles.

In the cat's ear Hooker (1920) failed to observe any influence upon the caliber of the capillaries and venules of section of the cervical sympathetic, but in the rabbit's ear Rehberg and I have seen them dilate definitely after the corresponding operation. In this case we have no direct evidence that the arterial dilatation resulting from the section might not be responsible, through the increase in capillary pressure, for the observed reaction, but we have other evidence, to be given later, that the capillaries of the ear are under the permanent influence of a strong sympathetic tonus. Like the visible arteries the capillaries of the rabbit's ear regain their

tonus a day or two after section of the cervical sympathetic, while the arterioles remain dilated for several days, as seen from temperature observations on the ears.

When the tonus is reëstablished after section of the nerves, we have observed in the frog that the regulation of the capillary tonus in the web is, as a rule, very imperfect. States of strong contraction alternate with more or less complete relaxation. Breslauer (1919) has made corresponding observations on patients with nerve lesions and mentions the "spontaneous" changes from ischemia to hyperemia and *vice versa*.

The tonic sympathetic innervation of capillaries is probably of very widespread occurrence in the vertebrate organism. There are, however, a few organs in which it seems to be absent. One of these is the tongue of the green frog (*Rana esculenta*), though the evidence is not conclusive. Electrical stimulation of the lingual nerves may cause contraction of some arteries but has no constrictor influence on capillaries, and, when the propagation of impulses along the nerves is blocked by cooling to the freezing point or actual freezing of the nerves, usually some dilatation of arteries occurs, but the response of capillaries is so slight that it is probably to be explained by the increased blood pressure to which they become subjected by the arterial relaxation. In view, however, of the later experience obtained on the web it seems probable that tonic impulses may reach the tongue capillaries mainly or exclusively via the arteries. The capillaries are well equipped with a net of non-medullated nerve fibers (Busch).

#### *Dilator innervation of capillaries.*

When the lingual nerves of a frog (especially the glossopharyngeus) are stimulated mechanically by



pinching, a distinct hyperemia is produced in the tongue, due to dilatation of capillaries as well as of arteries.<sup>1</sup> In a few cases the dilatation of the capillaries has preponderated to such an extent that the current of blood through them became visibly slower. This dilator effect develops after a latent period of several seconds or even one-fourth to one-half minute. It subsides very gradually during fifteen minutes or more. These vasodilator reactions call to mind the "antidromic" vasodilatation produced in the legs of mammals by mechanical stimulation of the sciatic and shown (Bayliss, 1901 and 1902) to be localized in posterior root fibers. Bayliss' experiments have given no definite proof that the capillaries are involved in his antidromic dilatations, and in the case of the frog's tongue there is no definite proof that the stimuli are propagated along posterior root fibers. In the case of the frog's hind legs, however, it can easily be demonstrated that stimulation of posterior roots will cause dilatation of capillaries as well as of arteries in the web and skin. This was first shown by Doi (1920) and confirmed by Krogh, Harrop, and Rehberg (1922), who found, however, that it is probably only a limited, though rather large, number of the capillaries in the web which will respond to stimulation of posterior roots. That the dilatation of arteries cannot be responsible for the capillary reactions observed was shown by Doi by the application of a dose of acetyl-choline. This drug will cause a maximal dilatation of the arteries and thereby prevent any response on their part to the nerve stimulation. In these circumstances the observed dilatation of capillaries can only be due to relaxation of their own tonus.

In the capillaries of frogs' muscles the antidromic dilator innervation appears to be slight and sometimes absent. Bayliss found on mammals that the dilator



effect of stimulating posterior roots, as measured by a pléthysmograph, was practically abolished when the leg was skinned. It should be remembered that very few posterior root fibers go to the muscles compared with the large number supplying the skin. According to the experience of surgeons (Busch) the visible arteries in human muscles are well equipped with the organs of pain while the muscular tissue itself—with the small vessels—can be crushed without giving rise to any sensation of pain.

In the case of mammals the proof that the capillaries are actively involved in the “antidromic” reaction is given by Lewis (p. 211), who has further brought forward very suggestive evidence that the dilatation due to stimulation of posterior root fibers is brought about through liberation in the tissue or in the walls of larger vessels of a specific dilator substance closely related to (perhaps identical with) histamine and called by him the H-substance of which we shall hear a great deal more in a succeeding lecture. Having excised the stellate ganglion of a cat and thereby caused degeneration of the sympathetic fibers to one fore limb, Lewis and Marvin stimulated peripheral branches of the median nerve to this paw and produced an “antidromic” dilatation of the vessels in the corresponding pads of the foot. They obtained a very conspicuous flush of the pads showing that the capillaries are directly involved. During a short faradic stimulation this dilatation gradually increases to a maximum over a period of 20 to 40 seconds of stimulation, to decline, when stimulation ceases, at an imperceptible rate over 5 or 10 minutes. The gradual appearance of the flush suggests a cumulative process, and the very gradual decline suggests the slow removal of a vasodilator substance (see Lecture X, p. 211).

The crucial test employed by Lewis was to stimulate

after occluding the circulation to the limb and to maintain the occlusion for a period equal to or surpassing that during which the reaction would otherwise subside (5-10 minutes). After such an occlusion the hyperemia would appear and be maintained in the stimulated pads after the subsidence of the general reactive hyperemia, and it was found to require, with remarkable exactitude, the usual time for fading away. This is, certainly, difficult to understand, unless we assume the formation of a vasodilator substance which remains in the tissue during the period of occlusion.

In the clinical symptoms of the disease known as Herpes zoster we have very definite evidence that a large number of skin capillaries and venules of the limbs, trunk, and at least the larger part of the head are in connection with posterior root fibers and become dilated when these are stimulated, probably mechanically, by pathological processes. Herpes zoster is characterized by such processes, usually of an inflammatory character, taking place in one or more of the spinal ganglia or in the Gasserian ganglion. The initial symptoms are dilatations of small vessels, including capillaries and venules in the zone directly innervated by the fibers from the ganglion affected, and the correspondence is so close that a reflex dilatation, produced by the pain usually associated with the initial stages of Herpes zoster, can be excluded. The only possibility seems to be a conduction of impulses along posterior root fibers to the skin vessels, and the evidence points to the conclusion that it is a general feature in vertebrates that vessels of the skin and notably a number of capillaries are connected with posterior root fibers, and that impulses traveling along these will cause dilatation of the vessels concerned through liberation of Lewis' H-substance.

Bayliss has shown by absolutely conclusive evidence

in the case of the mammals examined by him that the dilator fibers in question could not be distinguished from normal bipolar sensory fibers with their cells in the spinal ganglion connected both with the periphery and with the spinal cord. In the case of frogs we have found in my laboratory that no degeneration of these fibers takes place when the posterior roots are cut between the spinal ganglia and the cord.

Woollard (1927), who studied the distribution of medullated sensory fibers along the vessels of cats and rabbits by means of vital staining with methylene blue, came to the conclusion that these nerves supply directly the smaller arteries and arterioles in the walls of which they form a plexus independent of the sympathetic. On the capillaries no nerve endings of this category could be shown to exist, and in the arteries the endings were mainly to be found in the adventitial sheath, very few penetrating into the muscles, while numerous collaterals supply the tissue elements near the vessels. This would agree well with Lewis' contention that the dilatation is produced by release in the tissue of a substance which comes in contact with the contractile cells after diffusion. The "connection" of the capillaries with the sensory fibers must, therefore, be taken to be indirect.

It is extremely doubtful whether the antidromic innervation of vessels along sensory nerve paths is ever functionally active, in the sense that impulses from the central nervous system reach the periphery directly through posterior roots, which would involve that at least one set of synapses should allow the passage of impulses in both directions. Though Bayliss (1902) and Lewis (1927, p. 235) seem to be of opinion that an antidromic innervation in this *true* sense exists, the evidence appears to me quite inconclusive. In the frog, I, myself, have tried in vain to influence the state of

contraction of the small vessels reflexly through posterior roots as the efferent path. Horiuchi (1924), who finds that the larger veins can, like other vessels, be dilated by stimulation of posterior root fibers, has seen dilatation of a vein, after cutting and stimulating the central end of the sympathetic nerve supplying it. This would be evidence of a reflex having posterior root fibers as the efferent path—if the experiment had been sufficiently controlled. If their reality is established the stigmatization phenomena, briefly discussed by Lewis (p. 214), in which reddening and vesication of the skin are reported as being produced by suggestion, would be explained with least difficulty on the basis of true antidromic innervation of the vessels concerned. The possibility, discussed by Ranson and Wightman (1922), that cells in the spinal ganglion can receive impulses through sympathetic preganglionic fibers should not be lost sight of as a possible alternative to an antidromic innervation in the strict sense of that term. Synapses of the type postulated have been demonstrated histologically.

In accordance with the general conception of specific nervous energies—a conception which is, by the way, very difficult to maintain when carried to the last consequences—we have to assume the existence of separate fibers or systems of fibers subserving the different cutaneous sensations, viz., pain, pressure, heat, etc. We cannot assume that impulses traveling along the fibers of any one or other of these systems are able to influence the blood vessels and can be responsible for the symptoms of Herpes zoster. A very early subjective symptom of this disease is the itching and pain, localized in consciousness to the area where the vascular symptoms appear, and it is natural, therefore, to assume that the fibers transmitting the sensation of pain are those responsible for the vascular reactions.



In a very recent contribution Neuburger (1927) has brought forward evidence to show that stigmatization is produced by mental concentration on an imagined sensation of very severe pain, localized to the area which is to become stigmatized.

While I do not deny the *possibility* that impulses may pass antidromically from the central nervous system or even the brain through the pain fibers of the posterior roots to the blood vessels, I am certainly of opinion that such an exception to the general rule of "forward direction" in the nervous system requires direct and strong evidence to become acceptable. Such evidence has not hitherto been forthcoming, and we seem, according to the description so far given, to be confronted with the paradox of a nervous connection which cannot function naturally, but only when the posterior root fibers are artificially or pathologically stimulated.

It is scarcely necessary to emphasize the fact that our knowledge of capillary innervation is extremely imperfect. Only a very few organs have been studied in the frog and one or two mammals. Next to nothing is known about the innervation of capillaries in such organs as the intestines, the glands, or the central nervous system. They may or may not be subjected to tonic impulses from the dorsal sympathetic from which many of them certainly receive nerve fibers; they may or may not be provided with special dilator fibers belonging to the cranio-sacral or even perhaps in some cases to the sensory system. There is here a wide and probably very fruitful field for future research. The information at hand will enable us, however, to get some insight into the mechanism of certain vascular reflexes which I shall proceed to describe and discuss in the next lecture, and the study of these will be found



to extend and deepen our knowledge of the innervation both in the anatomical and the physiological direction.

## NOTE

<sup>1</sup> I have not succeeded in producing this capillary hyperemia by electric stimulation, but I find that Bruck (1909) has obtained it in one-half of the tongue by faradic stimulation of the corresponding glossopharyngeal nerve.

## LECTURE VI

### VASCULAR REFLEXES

#### *"Reflex Erythema."*

**W**HEN the tongue of a deeply narcotized frog (*R. esculenta*) is pinned out and a needle is drawn across its surface, a hyperemic zone 2-4 mm. broad will begin to develop after a latent period of a few seconds. In an anemic tongue a number of closed capillaries will be opened up and greatly dilated, as shown in the film, while the arteries supplying the hyperemic zone become dilated way back in the tongue.

The mechanism of this reaction has been studied by a number of varied experiments. That the spreading of the reaction from the line (or point) directly stimulated must take place along nerve fibers can be concluded from the time of latency and the rapid rate of spreading after the latent period. When the surface of the tongue is anesthetized by cocainization the reaction is abolished, indicating that sensory nerve endings are involved, and a reflex arc from these to spinal centers and back over sympathetic fibers would, therefore, normally be expected. As pointed out above, the sympathetic innervation of the frog's tongue is rather doubtful, however, and the possibility of a true spinal reflex becomes excluded by the fact that section of all the lingual nerves at their entrance into the tongue does not influence the reaction in the least. Only when, after a period of several weeks, the nerves have degenerated completely the stimulus fails to produce a hyperemic

zone and confines the reaction of the vessels to the line directly stimulated.

It follows from these facts that we have to do with a local nervous mechanism in which sensory nerve endings are probably involved (since cocainization will abolish the reaction) and also, of course, those fibers by the mechanical stimulation of which dilatation could be produced and which are, according to the evidence presented above, assumed to be sensory.

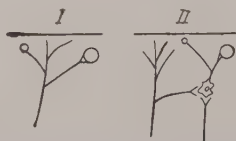


Fig. 42. Alternative diagrams of axon reflex.

Two different local mechanisms can be conceived and are illustrated by the diagram (Fig. 42). In I the single nerve fibers are supposed to split up into fibrils of which some are in connection with the contractile elements of the vessels. On stimulation of one or more of their hypothetical end organs the impulse will spread to all the branches of that nerve fiber and cause relaxation of the capillary and arterial muscle cells with which it is connected. This is the original conception of an antidromic local reflex adopted by Bruce (1910) to account for his observations on the conjunctiva of rabbits. The diagram II corresponds to a conception to which Bardy (1915) was led in studying the details of local vascular reactions to stimuli in the conjunctiva. It assumes a stimulation of sensory nerve endings which is carried through special branches of the fibers concerned to local vasomotor ganglion cells and from these on to the vessels.

The experimental results obtained on the frog's tongue can be best explained on the simpler assumption of sensory nerve fibers being directly connected with the contractile cells. On the hypothesis that the stimulus is carried to an autonomic ganglion cell one must expect that all the elements innervated from this cell would react simultaneously and to the same extent, and there would be no reason to expect that the point stimulated would always show the most intense reaction. It is possible, however, by very cautious mechanical stimulation of a single point, to restrict the response to a length of capillary corresponding to 2 to 4 Rouget cells and to graduate it from this minimum by very small steps to a maximum involving a capillary area of 5 mm.<sup>2</sup>, or more, and 5 to 10 mm. of artery supplying this area. We must assume, therefore, that the nerve fibers which accompany the smaller arteries and arterioles give off fibrillar branches to their musculature, before they are finally split up in a number of branches supplying the mucous membrane and its capillary vessels. A stimulus applied to one of the branches must travel in a central direction along this, but whenever it comes to a point of bifurcation it will be distributed between the stem and the other branch. Judging by analogy from what is known to happen in the nerve nets of lower animals we may suppose it to be weakened by the distribution and this assumption will explain in a simple manner the fact that the area to which the reaction spreads depends (at least roughly) upon the strength of the initial stimulus.

By repeated mechanical stimulation the reaction can be further extended, and chemical stimulation, e.g., by means of a small crystal of silver nitrate placed on the tongue, will give rise, according to experiments by Rehberg, to a distinct hyperemia involving a large part of

the tongue. The mechanism of this reaction is the same as of that due to mechanical stimulation, and the greater extension is probably brought about by the summation of the stimuli from the slowly dissolving corrosive substance.

Since the stimuli affect both arteries and capillaries the effect on the latter might be considered as secondary and due to the increase in pressure caused by the dilatation of arterioles. To test this point a small adjustable clip was arranged (Krogh, 1920) on the right lingual artery and the vessel compressed until the current of blood became very slow and the pressure, therefore, low. Mechanical or chemical stimulation on the right side had the normal effect, viz.: a number of capillaries over a considerable area opened up, blood flowed slowly into them and they became gradually so much dilated that several corpuscles could pass side by side. When the clip was opened the current through the capillaries became rapid; but no further dilatation of the open capillaries could be observed. Several capillaries became injected, however, which had not been visible before. This experiment has been repeated and varied, and there can be no doubt that the reflex affects capillaries as well as arterioles, while there is no evidence of any active relaxation of veins.

In the web and skin of the frog the reactions to mechanical stimulation are complicated and on the whole inconspicuous, but a considerable erythema can be brought about by chemical means. If a small crystal of silver nitrate is placed on the web of a frog all the arteries between the two toes, where the crystal is placed, and often a large number of those between neighboring toes will dilate, after a latency of about 10 seconds. A few seconds later a large number of capillaries will also show dilatation, which can be shown to be in the main independent of the increase in pres-



sure and blood flow resulting from the widening of the arteries. This reaction, like the corresponding one in the tongue, depends upon a local axon reflex which can in this case be shown to take its course through posterior root fibers. In our experiments it was not affected in the least by simple section of the sciatic nerve, but after degeneration, which required a very long time (80 days in one case), it became completely abolished. Section of the posterior roots of the ninth and tenth spinal nerves above their ganglia left the reflex mechanism entirely intact for the whole period of observation, while operative removal of the ninth and tenth posterior root ganglia, which was expected to abolish it after degeneration, only caused a distinct weakening after about 40 days, with a recovery toward the normal state after 100-150 days in a few frogs surviving for that length of time. In this latter case it is to be remembered that the removal of the ganglion cells cannot be complete.

In the skin of human beings a similar reaction has been observed from time immemorial which is now generally termed "reflex erythema," "reflex hyperemia," or "flare" (Lewis).

When a needle is drawn across the skin in man so as to provoke a more or less painful sensation, or when a sensation of pain is brought about in any other way, for instance, by the action of high or very low temperature or of a great variety of chemical agents, the stimulus will result after a short period of latency in a red area of irregular form and variable size surrounding the point directly stimulated. This point may itself show various reactions, according to the nature and strength of the stimulus. With these we are not now concerned, but we will confine our attention to the surrounding erythema. A microscopical examination of the erythematous skin and a comparison with

normal skin reveal the fact that the redness is due to a large number of skin capillaries and small veins which previously were closed, or at least very narrow, but are now open and comparatively wide. In these dilated vessels there is a rapid current of blood, showing that the arteries and arterioles of the affected area have also become dilated.

This reaction was formerly ascribed (Müller, 1913) to a spinal reflex having a very short path in the corresponding segment of the cord, but more recent investigations have shown it to be due mainly, if not exclusively, to an axon reflex in sensory fibers, strictly comparable to that observed in the frog's tongue or skin.

Breslauër (now Schück) studied in 1919 the reactions of human skin to mustard oil, which produces on normal skin a painful sensation and a considerable hyperemia, spreading over a larger area than that which has been in contact with the mustard. On applying mustard oil to the anesthetic skin in patients, in whom the corresponding nerves had been damaged and had degenerated, no reaction whatever could be observed. When in a skin area a trace of pain sensibility was left a reaction would also take place, which was in some cases almost as strong as on the normal skin of the same person. In experiments on his own skin, anesthetized with novocaine, no reaction appeared, until the novocaine ceased to act, and an erythema developed *pari passu* with the pain felt. When the nerve trunk was anesthetized, or in patients with freshly damaged nerves, it was found that the reaction developed normally and had nothing to do with any *sensation* of pain which was, of course, absent in these cases.

Kohler and Weth (1924) have seen a human case in which the reflex erythema developed normally after de-

generation of the postganglionic sympathetic fibers to a limb and have thus proved its localization in sensory fibers.

Lewis and Grant (1925) and (somewhat later) Török and Rajka (1925) have studied the reflex erythema following mechanical, thermal, and chemical (histamine) stimulation and confirm the findings of Breslau. In one case, described by Lewis and Grant, the reaction was unchanged 14 days after the corresponding nerve trunk had been injured, but was found to be absent 7 days later.

The axon reflex spreads in the human skin to a very considerable distance. The visible limit of the flare is usually determined, according to Lewis, by the tone of capillaries and venules, so that it will be bordered by pale areas having a high tone and being at a distance of some 2-3 cm. from the point stimulated. Areas in which the tone of the minute vessels is weakened (e.g., by light or other injury, cf., p. 216) may flare up, however, at much larger distances. Lewis has seen flares of 6-7 cm. radius and mentions a case in which a formerly injured area lighted up at a distance of 17 cm. from the point stimulated. It appears to me necessary to conclude from these observations that the spread of the reflex normally involves a much larger area of the skin than that which actually flares up. In the remaining part the flare is either inhibited by a reaction in the opposite direction or simply weakened by decrement in the nerve fibrils.

The detailed mechanism of the axon reflex flare has been very carefully studied on the human skin by Lewis and his collaborators. On most points I can subscribe to their findings and conclusions, but there are a few on which I have to disagree. Lewis describes the flare as arteriolar and is of opinion that it is localized exclusively in his so-called "strong" arterioles, while

the smaller vessels, those arterioles, capillaries, and venules which are designated by Lewis as the "minute vessels" are only passively expanded.

This conception is at variance with the observations made in experiments on frogs and is very hard to reconcile with the general conception of blood flow regulation maintained in these lectures, and it is necessary, therefore, to examine the evidence in some detail.

As pointed out in a preceding lecture (IV, p. 98), the anatomical distinction between "strong" arterioles, possessing a distinct muscular coat, and "terminal" arterioles, supposed to be purely endothelial, cannot be maintained. The wall is muscular in all of them.

That blood flow is increased in the hyperemic area and that arterioles must, therefore, be involved is concluded by Lewis from the distinct increase in temperature of the area as compared with normal skin (cf., p. 156) and, further, from the fact that on an arm made bluish by venous congestion an area of flare will stand out conspicuously in a bright arterial color. The arteriolar dilatation is taken to be sufficient to expand the "minute vessels" supplied by them by the increased pressure to which they become subjected. It is observed that after occlusion of the circulation to an arm the flare fails to develop when an adequate stimulus is applied, but is rendered visible and distinct when the reactive hyperemia that follows the opening up of the circulation has subsided. This is taken to prove that there can be no active relaxation of the minute vessels responsible for the color of the skin, since, if relaxed, they would take up blood from the surroundings and cause a visible flare *during* the occlusion. Lewis concludes finally from the irregular outline of the flare, frequently showing indentations and isolated islands of one to a few mm. diameter and which

can be often exactly reproduced by renewed stimulation of the same point, that the smallest vessels involved in the reflex dilatation must be arterioles supplying areas of a few mm. diameter. This points to the "arched arterioles" connecting the subpapillary with the deeper cutaneous plexus of arteries.

While Lewis' observations that arterioles are involved and the blood flow considerably increased in the reflex flare are certainly conclusive, the arguments against the participation of the capillaries and venules are to my mind quite unconvincing. According to Lewis' own observations, which I can confirm, a pressure of 20-40 mm. of mercury obtained by congesting the veins in an arm does not cause any dilatation of the venules comparable to that seen in a reflex flare; it is inconceivable that the pressure can be locally raised to such an extent in venules anastomosing freely and having numerous open connections with larger veins in which the pressure cannot exceed a few mm., and direct measurements, to be given later, show that it is not so raised. It is well known, further, especially from observations on skin temperatures, that the blood flow can be very greatly increased without causing any visible increase in color of the skin. Detailed evidence will be given later, and it will be shown that a flow of almost any magnitude brought about by dilatation of arterioles can be taken up by capillaries and venules without any appreciable dilatation.

We must conclude, therefore, that arterioles, capillaries, and venules are alike involved in the reflex flare and become actively relaxed. As we shall see, this is of some importance from the point of view of the nervous mechanism involved.<sup>1</sup>

Concerning the exact path of the reflex the evidence of the literature is somewhat conflicting. Török and Rajka (1925) state that a deep subcutaneous injection



of novocaine which produces local anesthesia without reaching the true skin does not affect the reaction which would, therefore, seem to be brought about through fibrils and fibers running in or directly above or below the corium.

Lewis, Grant, and Marvin (1927), without directly contradicting Török and Rajka, state emphatically that an intradermal barrier of novocaine does not prevent or even affect the spreading of the flare beyond. In other experiments they made incisions of about 2 cm. length through the skin to a depth of 3-4 mm. and saw the flare spread beyond them to the exact limit reached in similar experiments before the incision. These results point in their opinion to the subcutis as the seat of division and horizontal distribution of the nerve fibers involved.

In view of the fact that the nervous impulses spread far beyond the visible limits of the flare it appears possible, however, that they pass round the ends of the simple barriers or incisions established by Lewis and his collaborators, and such a passage is to be definitely expected if the impulses spread in a close-meshed continuous network of fibrils. In such a net the spreading can be prevented only if the point stimulated is surrounded on all sides by barriers or incisions. We have in my laboratory made such a crucial experiment. The constancy of the flare produced by pricking in strong histamine at a suitable point on the breast of P. R. was established by a few repetitions and four incisions were then made through the whole depth of the true skin surrounding the selected spot. Making a histamine test on the sixth day after the operation it was found that the histamine in the isolated piece of skin caused almost intolerable itching, while a control spot gave the normal sensation. Whealing was normal and a strongly red flare was produced within the field with a

number of faintly red spots outside and mainly laterally (Fig. 43). Histamine pricks laid down outside the field (seventh to eighth day) failed to produce any reaction within and gave a practically normal sensation. On the tenth day the sensation from a histamine prick inside the field was more nearly normal and the reaction outside barely visible. Some regeneration appears to take place quickly. On the nineteenth day the incisions showed a bright red scar. Histamine pricks within gave a definite reaction laterally on the out-

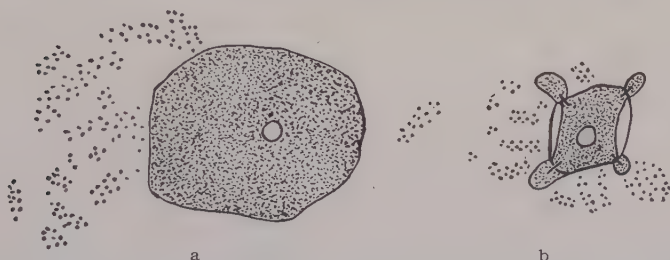


Fig. 43. Flare surrounding histamine prick before (a) and 6 days after (b) isolating the site by 4 cuts just penetrating the skin. The closely dotted areas are uniformly red, the openly dotted show a faint and discontinuous flare.

side, but none medially, and pricks outside on the lateral side produced a distinct flare within, while none could be provoked from the medial side. The sensation was normal in all cases. We have to conclude that most of the paths of the reflex were cut by the incisions, while a few probably deeper connections remained.

As far as I have been able to find Lewis expresses no opinion in his book with regard to the kind of sensory fibers involved in the "reflex" flare, but in the recent paper by Lewis and Marvin the opinion is recorded that we have to do with special fibers which are distinct from those shown to produce H-substance when stimulated antidromically, which give rise to the spe-

cific sensation of itching and which are capable of being stimulated only chemically by H-substance. According to Lewis we would have to picture a system with numerous receptor endings in the epidermis and adjacent parts of the cutis and with effector endings not reaching farther out than to the "strong" arterioles in the deeper layers of the true skin.

I am unable to accept this picture as consistent with well-established facts, and the evidence brought forward appears to me inconclusive. It seems to me natural to assume that we have one set of fibers—those transmitting pain—which liberate H-substance from their endings when suitably stimulated either in the spinal ganglion (Herpes zoster), along their course (antidromically), or in the periphery (producing "reflex" flare).

I am not prepared to discuss the relation of itching to other sensations. Subjectively I find the itching produced by histamine in any concentration extremely slight compared with what is felt after mosquito bites or introduction of nettle poison, while that produced by certain tropical nettles is again much stronger. I take itching to be due mainly to stimulation of pain fibers.

The conclusion that no H-substance is produced in the area of the flare, and that the fibers cannot, therefore, be the same as those conducting "antidromically," is derived by Lewis from experiments, in which flares were partly covered by an Esmarch bandage as shown in Fig. 44. When the three histamine pricks *a*, *b*, and *c* were laid down simultaneously, and the bandage applied after the flares had developed and removed after 15 to 20 minutes, when *a* and the uncovered portion of *c* had diminished considerably, it was found that the histamine in *b* was retained by the bandage and would maintain the corresponding flare for a

definite period after the passing off of the reactive hyperemia due to circulatory arrest (discussed in detail in Lecture X, p. 223), whereas in *c* the covered portion as well as the uncovered portion of the flare would disappear *pari passu* with the control. This result certainly establishes a difference between the behavior of the histamine deposited in the pricks and that of the H-substance which I suppose to be liberated from nerve endings and distributed in extremely low concentration over the whole area of the flare, but it

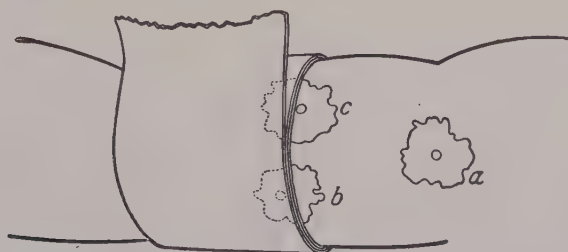


Fig. 44. Histamine pricks on upper arm with surrounding flares partially covered by Esmarch bandage.  
After Lewis, Grant and Marvin.

does not show that no substance is liberated in the flare area. It is extremely natural to suppose that the flood of the reactive hyperemia is sufficient to remove such a substance and dilute it below the threshold, especially along the vessels.

Lewis, Grant, and Marvin (1927) make precisely this assumption to account for the behavior of the flares produced by faradic stimulation. They show that faradic currents like other stimuli (Lecture X) act indirectly by causing the liberation of H-substance over a certain area. They find that this H-substance gives rise to a widespread flare, but when they repeat the experiments of holding up the flare by circulatory ar-

rest the attempt fails as often as not. They state that "this failure would seem to indicate that the released substance responsible for the flare is more susceptible to quick removal by the flood of reactive hyperaemia than is the case where other forms of stimulation are concerned" and they suggest that this is due to the substance being released in weak concentration over a wide area.

I find no definite evidence, therefore, against the assumption that the path of the reflex erythema is along fibrils and fibers of the pain nerves, and the observations of Breslauer quoted on page 128 point very definitely to these. The relation of the reaction to the pain *sensation* is, however, complicated and somewhat obscure. Lewis maintains (p. 216) that there are forms of stimulation (weak galvanic stimulation, chemical irritants such as mustard gas) which will evoke the flare without awakening a localized sensation of any kind.

It should be remembered that pain sensations, more even than other kinds of sensation, are determined not only by the stimuli at the periphery, but by conditions in the central nervous system which are empirically well known although very imperfectly understood. It can be said generally that a brief stimulus, which will evoke a sharp sensation of pain from the skin, is insufficient to cause the formation of a surrounding flare, while a prolonged weak stimulation, which is felt as pain only when attention is focused on the sensation, will readily do so. If we accept, as I think we must, the conclusion that the nerve endings act through the formation of Lewis' H-substance it seems natural to assume that this will require a prolonged stimulation. I find no difficulty in assuming that a stimulation of pain fibers may increase so slowly and remain so weak that no sensation reaches consciousness, while suffi-



cient H-substance is released at the nerve endings to produce a flare.

A further discussion on the relation of the flare to the H-substance will be given on p. 211.

While it appears impossible to doubt the validity of the conclusion that we have to do in the cases of reflex erythema so far discussed with an axon reflex localized in peripheral fibrils and fibers of sensory nerve paths, mainly and perhaps exclusively in those fibers transmitting pain, a number of the experimental results further point to a definite new conception regarding a peripheral distribution and interconnection of these fibers, at variance with the commonly accepted idea that each neurone is a separate entity, viz., that all the fibrils from a number of fibers are connected up to form a continuous peripheral network, in which conduction takes place with a decrement at every point where an impulse traveling along one of the fibrils has to distribute itself to two or more threads in the net and a corresponding increment whenever these impulses meet again at other points in the network. Any alternatives to this conception are very hard to reconcile with the facts. The wide extension of the reflex erythema could be explained, if we assume that each neurone spreads over a considerable area, and, further, that there is a great deal of overlapping so that every square mm. of the skin is supplied by several neurones, each of which supplies independently an area (in the human skin) of at least several square centimeters. Such an assumption is intrinsically improbable, but is rendered practically untenable by the incision experiments made by Lewis, Grant, and Marvin combined with those made by Rehberg and myself. In several cases Breslauer observed further that the fibers connecting a certain area with the brain had degenerated to such an extent that only the slightest

pain could be felt, but in spite of that a normal reflex erythema could develop. On the neurone theory we should expect in such cases a very faint reaction (because very few neurones could be involved) and the existence of at least some spots where no reaction could be obtained. On the theory of a peripheral fibrillar net we must make the further very probable as-



Fig. 45. Histamine (1 in 300) was punctured into the skin of the forearm at *A* and yielded the flare outlined by the continuous line *a*. The same spot was repunctured 3 hours later with a 1 in 30 solution with a precise repetition of the flare. Two hours later point *B* was punctured with a 1 in 30 solution; the flare appeared over the area outlined by the dotted line *b*. Several hours later the intermediate point *C* was tested similarly. The resultant flare precisely filled the outline *a*. After Lewis.

Fig. 46. Histamine (1 in 30) was punctured into the point *W* on the forearm, whealed the skin and produced the flare outlined by *w*. After 4 minutes the wheal began to show a lymphatic extension which lengthened gradually. When it had lengthened to about 1 centimeter, a new area of flare *p* appeared abruptly. After Lewis.

sumption that a very small number of fibers connecting the net with spinal ganglion cells is sufficient to prevent degeneration of the net.

A number of experiments described by Lewis (p. 218) are seemingly at variance with the theory of a nerve net and point to the involvement of distinct neurones. Lewis found that a certain strength of histamine punctured into the skin would produce a maxi-

num flare which would be exactly reproduced when later the same or a stronger solution was punctured in at the same point or very close to it (A and C in Fig. 45). Shifting the point of repuncture to B caused a definite new area to be included, while part of the old was left uninvaded. A sudden invasion of a new area caused by histamine diffusing along a lymphatic vessel is shown in Fig. 46. Lewis points out, however, that the extension of the flare by steps which is so strongly suggestive of the involvement of a new neurone is in reality due to a wholly different cause, namely to local differences in the tone of the minute vessels in adjacent and defined areas of the skin. By examining carefully the color of the skin he has been able to predict with considerable accuracy the borders to which a subsequent histamine flare would probably reach.<sup>2</sup>

When the flare subsides the minute vessels regain their tone. This takes place in irregular patches, and areas which were pale beforehand are usually the first to lose color and to become often distinctly more pallid than before. As assumed by Lewis, this may possibly be due to a response on the part of the small vessels to the increased supply of blood received, but Rajka and Fürth (1924) assume that we have, superimposed upon the dilator axon reflex in posterior root fibers, a constrictor reflex in sympathetic fibers of the type which will presently be discussed, and this is at least a possibility which should also be kept in mind.

Axon reflexes of the type responsible for the flare of the human skin are of widespread occurrence in mammals generally. The reaction appears to be especially well developed in the conjunctiva (Bruce, 1910; Breslauer, 1919). Breslauer has observed the reaction in patients in the parietal peritoneum and parts of the mesentery, notably along the larger vessels, where it coincides with the distribution of nerves conducting

pain sensations. It cannot be provoked from the gut and the mesentery nearest to it, and according to Ebbecke (1917) it is absent on the surface of internal organs like the liver or kidney. It is also, according to Florey (1925), absent from the pia mater of the brain. In the ears of rabbits the axon reflex mechanism is found as a rudiment, while reactions of quite similar appearance are brought about as true reflexes to be discussed in the next lecture.

### NOTES

<sup>1</sup> The occlusion experiments are complicated by the fact that during the occlusion all the small vessels in the occluded arm relax, while the blood becomes venous as discussed in detail in a following lecture (p. 223). Even when the vessels relax somewhat more in the flare area, they have no possibility of drawing to themselves much blood from the surroundings and it is to be remembered that vessels filled with venous blood stand out much less distinctly than those of an arterial color. All that can be expected is a faint flare containing slightly more blood of the same bluish color as the surrounding skin. As a matter of fact such a "ghost-like flush" first described by Rehberg and Carrier (1922) has been often observed by Lewis himself. We (Rehberg and myself) have now repeated the occlusion experiments and find that the appearance of a faint bluish flare during occlusion is the rule rather than the exception on white arms. In several cases the outline was found to correspond closely to the succeeding arterial flare, in others the bluish area was distinctly smaller. In my opinion these observations can only mean that the capillaries and venules are directly involved in the reflex relaxation. In his latest publication (1928) Lewis denies the reality of the ghost-flare.

Lewis himself describes how a well developed flare fails to disappear when the arterial pressure is reduced to 0 by occlusion and how the blood, when mechanically removed during occlusion of the artery from the dilated vessels, will return slowly from the surrounding veins, which appears to be possible only when the vessels are relaxed, but certainly not when they have been simply distended by arteriolar pressure. In the corresponding reaction in a rabbit's ear, which can be studied in detail microscopically, it can be observed that capillaries dilate before the arterioles supplying them, but, of course, the mechanism might be different in the human skin.

Lewis admits (p. 147) that in skin which is pale and hot the arterioles are open while "the minute vessels have a high tone." If a histamine prick is laid down on such skin it should fail entirely to produce a flare.

Lewis argues on several occasions (1927, p. 24. Cotton Slade and L.

1917) from the precision with which a reaction in the skin can be delimited to the size and kind of vessels involved. These arguments are valid only with certain restrictions.

When the white reaction produced by gentle stroking with a flat ruler (see Lecture VIII, p. 163) is delimited by straight lines, corresponding exactly to the border of the area touched by the ruler, it is legitimate to conclude that vessels supplying areas of less than 1 sq. mm. must be involved. The limitation cannot, therefore, be explained by the exclusive involvement of larger arterioles than those of the subpapillary plexus, but it is evident that smaller as well as larger vessels, capillaries, veins, and larger arterioles may, so far as this observation is concerned, very well take part in the reaction. The state of filling of the larger and deeper vessels does not in any case contribute perceptibly to the skin color.

In the case of the limitation of the "arteriolar" flare what can be legitimately concluded from the crenulated appearance of the border line is that the reaction cannot be explained by the exclusive dilatation of larger vessels than the "arched" arterioles, but I fail to see why smaller vessels should not take part. The small vessels are able to account for a geometrically regular border line to a reaction, when the stimulus is so delimited, but there is no reason why a stimulus spreading through nerve paths from a point selected at random in the skin should spread to any geometrically defined limit. One would rather expect the spreading to be irregular. In certain individuals we find as a rule, outside the continuous flare, a number of distinct red spots from less than one to over 20 mm.<sup>2</sup> in size.

I have dealt at such length with this point of controversy between Lewis and myself, because it is fundamental for my general conception of capillary as distinguished from arterial control of the circulation.

Lewis maintains on several occasions and explicitly in a discussion on p. 33 of his book that arteriolar pressure may penetrate in full force to the net of subpapillary venules, while I hold that arterioles (and a fortiori larger arteries) cannot by their dilatation raise the pressure in the capillaries and venules to any appreciable extent. The capillary bed is always so much wider and the outflow through the venules and veins normally so free that the pressure varies but slightly and remains essentially low, even when the flow is greatly increased through arteriolar dilatation. A full discussion of pressures in the capillaries and veins will be given in a later lecture.

<sup>2</sup> The conception of peripheral nerve nets, each made up of the richly anastomosing fibrils of the corresponding nerve fibers, is, of course, so novel that it will have to be tested in several ways, both histologically and physiologically, and found confirmed, before it can be accepted as anything but a provisional working hypothesis. Personally I believe, however, that it will prove to be a fruitful hypothesis, and I would like to suggest that it might be worth while to study the cutaneous senses in man on the basis of the existence of such networks connecting up all the



sense points of each special sense and determining the "local sign" of any sensation by the *combination* of fibers, the "*conductive pattern*" (Sherrington, 1920, p. 179) through which the impulse is carried up to the brain.

The beautiful experiments and observations of Trotter and Davies (1909) undertaken on their own skin after section of large cutaneous nerves show that there are, surrounding the area in which all cutaneous sensation is lost, zones in which the specific sense points show a progressively diminished sensitivity (a higher and higher threshold) toward their adequate stimuli. These observations can be explained by assuming that each sense point is connected with a certain number of independent neurones passing through different nerve twigs to the larger trunks. To account for Trotter's and Davies' results the number of neurones to each sense point could not possibly be less than 3 and would probably have to be between 5 and 10. The assumption of peripheral fibrillar nerve nets appears to me much more natural.

The recent observations of Lewis and Marvin (1927, 3) point strongly to the existence of special nerve nets of limited extent formed by the pilomotor fibers.

## LECTURE VII

### VASCULAR REFLEXES (continued)

#### *Axon reflexes in the sympathetic system.*

WHEN in the spread web of a frog, examined at a low power with the binocular microscope, one of the arteries is pricked with a fine needle, it will generally contract over a length of several millimeters and often to complete obliteration. If the artery is hit and pierced the biological significance of this reaction reveals itself beautifully. After a few seconds latency the artery becomes closed and remains so for many minutes, and when it opens again the drop of blood shed through the wound has clotted and further bleeding is prevented. This reaction to mechanical injury is often observed also on larger arteries during operations on man and mammals. It is not confined to arteries, but is shown also by capillaries both in the frog and in man (Heimberger, 1925, 1). In the capillaries the distance to which the reaction spreads is, however, generally so short—a few tenths of a millimeter—that it seems doubtful whether we have to do with a nervous reaction, and I shall deal only with the mechanism of the arterial contraction which has been studied in the frog.

Considering the fact that the contraction may take place simultaneously over the whole length of a web artery, after so short a time that diffusion of a substance affecting the arterial musculature is out of the question, there can be no doubt that the reaction is propagated along nerves. It is not a true reflex, since

cutting of the sciatic or even removal of the whole of the nerve from the knee upward, including the sympathetic ganglia VIII to X, does not immediately influence it in the least. In animals surviving after this operation the reaction becomes weakened for a variable period between the fiftieth and hundred and twentieth day, but is never completely abolished, and further experimentation has shown that the weakening is due to removal of the sympathetic ganglia, while cutting of the anterior or posterior nerve roots is without any effect. The fact that no operation appears to be able to abolish the reaction points to the existence of aberrant ganglion cells, probably in the wall of the aorta or femoral artery, which are capable of preventing degeneration in the nerve nets along the arteries.<sup>1</sup>

*Long path axon reflexes.*

The axon reflexes so far dealt with have been strictly local. Their paths have been the fibrillar branches or the fibrillar network into which the nerve fibers ultimately split up. Evidence has been accumulating, however, indicating the existence of long path axon reflexes in sympathetic fibers playing an important part in vascular reactions.

The concept of the axon reflex was originally developed by Langley (1900) who found that stimulation of central stumps of certain nerves (e.g., N. hypogastricus) would provoke reactions at a distance (e.g., contraction of vessels in the anal mucous membrane). Langley showed that these reactions were not true reflexes, but were due to the branching of preganglionic fibers in the sympathetic system which supplied ganglion cells in several different places. Langley's preganglionic axon reflexes can be used to great advantage to study the distribution of the branches in question, but it should be borne in mind that they are

purely artificial: So far as we know there is no possibility in normal life that one particular branch of a preganglionic fiber can be independently stimulated and transmit the stimulus to ganglion cells at a distance. The axon reflexes with which we have to deal are natural reflexes and must be supposed to have a definite, although in many cases a very obscure, function in the economy of the body.

These long path axon reflexes have been specially studied in my laboratory by Wernöe, who endeavored to elucidate the reactions observed in patients suffering from internal disease. It is well known that diseased conditions in organs like the intestine, kidneys, lungs, etc., give rise to sensations appearing to arise from definite regions of the skin and to hyperalgesia on stimulation of these regions. The mechanism of these sensory disturbances was very obscure, but they were generally ascribed to central processes of "irradiation," while the skin itself was thought to be quite normal. Wernöe found in his studies of such patients (1920) that the hyperesthetic skin areas can be objectively demonstrated by their reactions to stimuli. When, for instance, the skin of the trunk is exposed to room temperature the blanching normally occurring is more pronounced in a hyperesthetic area than in the rest of the surface. To the application of heat the hyperesthetic zones respond with a more pronounced hyperemia than does the normal skin and the reactions to mechanical stimulation are also somewhat increased. These observations made the conception of purely central processes in sensory nerve cells practically untenable, and Wernöe (1925) undertook a search for nerve paths connecting each internal organ with the skin of the segment to which it belonged.<sup>2</sup>

The choice of experimental animals was difficult, but Wernöe found that very definite reactions could be ob-

tained from the chromatophores in the skin of fishes, while special experiments and observations showed that these reactions run parallel to those of the small blood vessels which are, however, much more difficult to observe. The skin chromatophores possess sympathetic innervation and contract strongly on application of adrenaline, as shown in Fig. 47, and on electric stimulation of the sympathetic.



Fig. 47. Retraction of cutaneous pigment in cod after application of adrenaline. After Wernöe.

In several species of fish (plaice, eel, and cod) Wernöe stimulated internal organs, especially the intestine, in various ways—chemically, electrically, and mechanically,—and observed blanching of the corresponding cutaneous segments and fibrillation of the muscles. Destruction of the spinal cord abolishes the fibrillation (which is brought about by a normal reflex) but it does not influence the cutaneous blanching. When nicotine is introduced into the circulation a general blanching is observed after a few minutes, but this is soon followed by a considerable darkening of the skin. The chromatophores are paralyzed at this stage, and their reactions to stimulation, direct or indirect,



are abolished. The initial blanching as well as the subsequent darkening are due to the action of the nicotine on the sympathetic ganglion cells, and painting of single ganglia with nicotine gives a corresponding reaction in the segment or segments concerned.

When the ganglia have been so painted stimulation of preganglionic sympathetic fibers must, of course, remain without any effect, and this has been used in Wernöe's experiments as a test to exclude the possi-



Fig. 48. Retraction of cutaneous pigment after stimulation of intestine. After Wernöe.

bility of preganglionic axon reflexes. The paralysis of the ganglia undoubtedly weakens the viscerocutaneous reflex, but strong chemical stimulation of the intestine of the cod will still bring about a blanching of the skin as shown in Fig. 48. Results such as this can be explained, as far as I can see, only by assuming that the nerve fibers from sympathetic ganglion cells divide and send branches to the intestine as well as to the skin, so that we have to do with typical postganglionic axon reflexes. This conception has been further strengthened by Wernöe in experiments showing reac-

tions in the intestine brought about by chemical stimulation of the skin. Wernöe describes experiments on the eel in which, after destruction of the brain and medulla, intensive chemical stimulation of the skin would bring about a strongly hyperemic condition of the intestine. After destruction of the spinal cord the same stimulus gives rise to a pronounced ischemia of the same organ, and it is possible on the same specimen to have both reactions side by side with sharp boundaries, as the segments in this form are very well defined. While the hyperemia is probably brought about by a true reflex<sup>3</sup> the contraction of the vessels after destruction of the spinal cord can only be due to the sympathetic axon reflex. The constrictor reflex skin-intestine has also been demonstrated by Wernöe in experiments on the cod and on the frog, but the segmental boundaries are in these animals difficult to make out.

Wernöe applies his results to the explanation of the phenomena observed in hyperesthetic zones in man. Although it is very probable that such direct axon connections between the internal organs and the skin exist also in man, and are involved in the reactions observed, the mechanism of these is still obscure.

Independent evidence for the existence of long path axon reflexes affecting the small blood vessels has been brought forward in experiments on frogs by Spersanskaja-Stepanowa. Finding evidence for such reflexes in the study of the secretion of skin glands (1925, 1, 2) she studied further the reactions of arteries and capillaries in the web to electric stimulation of distant parts of the same and the opposite leg. In these experiments the spinal roots were divided and also the dorsal sympathetic above the seventh ganglion, and time was allowed for degeneration. In these circumstances, where the only nerve paths available

were the postganglionic fibers of the eighth and ninth sympathetic ganglion, stimulation produced contractions of arteries and capillaries. The time of latency increased with the distance.

After degeneration of all nerves except the postganglionic fibers of the eighth segment reactions could only be provoked by stimulation of skin areas belonging to this segment. These reflexes were not immediately affected by cutting the sciatic high up in the leg, but after some days the reactions became diminished and could finally be produced only by stimulation at a short distance (as in the experiments described above, p. 143). The explanation given by Speranskaja-Stepanowa, and apparently the only one possible, is that the postganglionic sympathetic fibers branch freely and supply vessels and glands over large areas.

Certain observations point definitely to the existence of a sympathetic fibrillar nerve net in the web. After section and degeneration of the nerves at the base of the fourth toe no contraction of the arteries of this toe can be elicited by stimulation of its tip, while stimulation of the tip of the third toe will bring about contraction. This reaction is abolished by crushing the web in the middle between the third and fourth toe.

In a very interesting paper Albert (1924) has shown that vascular responses due to long path axon reflexes can also be elicited in the hind legs of dogs, especially by stimulation of the joints and their immediate surroundings, but as these reactions have not been shown to involve capillaries, and as the nature of the fibers concerned has not been ascertained, the results cannot be discussed here.

#### *True reflexes involving capillaries.*

I have dealt at such length with axon reflexes of different types that you might be led to think that these

were the only or at least the chief ones affecting capillaries. This is certainly not so, however. Regular reflexes of the spinal type are responsible for a large number of reactions, and it is possible here only to select a few examples. The afferent paths in these reflexes are sensory nerves of different kinds, while the efferent fibers, at least in all cases that have been studied more closely, belong to the dorsal sympathetic. The reflex mechanism is either an increase or a decrease in the sympathetic tone of the vessels involved. In the latter case the reflex is classed as inhibitor.

A reflex of this type was first studied by Lovén (1866) who found that artificial stimulation of the central end of the divided chief sensory nerve to the rabbit's ear caused a very pronounced vasodilatation in the ear, which could be prevented by cutting the cervical sympathetic. This reflex has been further studied by Rehberg and myself, applying adequate stimuli to the surface of the ear.

It is significant that we have here the double mechanism of a true reflex, combined with a local axon reflex in the sensory fibers themselves, this latter contributing, however, very slightly to the reaction. A strong mechanical stimulus produces on the intact ear a hyperemia stretching to a distance of one to several cm. and sometimes involving the whole of the ear surface. On an ear made anesthetic by cutting the sensory nerves, the axon reflex, which remains, is so slight as to be barely visible to the naked eye. Cocainization of an area abolishes both the true and the axon reflex. Chemical stimulation brought about by pricking in histamine is generally unable to provoke the true reflex, but the axon reflex can be observed though it is generally so slight as to be doubtful to a naked eye inspection.

Mechanical stimulation under the microscope with a

very fine needle or a hair will cause immediate dilatation of the capillary loop stimulated and, after a latency of a few seconds to half a minute, depending largely upon the temperature of the ear, dilatation of surrounding capillaries within a distance of about 1 mm., and finally of the artery supplying these capillaries. The capillaries are seen to dilate, and afterward the current through them becomes rapid. The strength of stimulus necessary to bring about this reaction is so small that it can scarcely be felt at all on the human skin, and no reflex erythema develops. The reaction is due to the local mechanism, since it is not immediately affected by section of the nerves to the ear. With the progress of degeneration in the divided (chiefly sensory) nerves the reaction becomes definitely weakened after twelve days and appears to be completely absent after nineteen days. I do not think that this reaction, which is so slight that it can be observed only by means of the microscope, has any functional significance, but would rather consider it as a physiological rudiment.

Constriction of skin capillaries as a response to the application of cold to distant parts of the skin has been observed repeatedly. Thus Otf. Müller (1922, p. 62) describes an observation by Weisz of the contraction even to obliteration of the nail fold capillaries when a piece of ice was applied to the arm. Lewis has seen diminution in volume of the arm both on the same and the opposite side on soaking one hand in cold water, but there is no definite proof that the minute vessels were involved. The efferent path for these reactions has been shown to be the sympathetic, and in the arms they are abolished by removal of the lower cervical ganglia.

A very curious and apparently very complicated reflex has been studied by Wernöe (1927) on 1,500 hu-



man subjects and is called by him the naso-ocular reflex. When a weak stimulus, such as the touch of a very soft brush, is applied to the mucous membrane of the nose the conjunctiva becomes hyperemic with opening up of a number of closed capillaries. As a rule stimulation of one nostril provokes the reaction equally in both eyes, but sometimes the reaction is weaker on the opposite side and in a certain percentage of cases (about 5 per cent) it appears only on the same side. The reflex path must be taken to pass the bulbar centers, since it is generally abolished in cases of bulbar paralysis. The afferent path has been found to run through sensory branches of the trigeminus nerve. L. R. Müller (1913) who has briefly discussed this reflex took the efferent path to be parasympathetic (cranial autonomic); but Wernöe rejects this hypothesis, because the reaction is not affected in the least by atropinization of the eye. The efferent path runs part of the way through the proximal part of the facial nerve, but reaches the eye finally through the first branch of the trigeminal, and this appears to exclude any possibility of an antidromic innervation through purely sensory neurones. We are brought by exclusion to the assumption of a sympathetic efferent path. Since we observe dilatation and not constriction of vessels the reflex appears to be one of sympathetic inhibition.

The matter is more complicated, however. The reflex is accompanied by a definite sensation (like that caused by a draft of air) and is abolished by cocainization, and to account for this Wernöe assumes that the sensory nerve endings (pain receptors) receive the sympathetic stimuli and bring about the reaction through the local axon reflex mechanism described above. This idea may seem far-fetched, but it is known that many primitive sense organs are supplied both with a medullated and a non-medullated fiber, and Orbeli (1926)

quoted by Brücke (1927) has shown that in the tongue of the dog these non-medullated fibers are sympathetic. It seems also necessary to assume a sympathetic innervation of the pain receptors in the skin in those cases of cutaneous hyperesthetic zones discussed above.

Evidence is given by Wernöe to show that in those cases, in which the naso-ocular reflex is unilateral and uncrossed, we have to do not with an isolated symptom, but with a more general anomaly in the sympathetic system which seems to be split in two more or less independent halves. Speranskaja-Stepanowa has observed the same anomaly in a certain number of the frogs examined by her (1925).

The naso-ocular vasodilator reflex has been found by Wernöe to be well developed in the dog, very weak in the horse, and absent in guinea pigs, rabbits, and ruminants.

#### *Psychic vascular reactions.*

Certain reflexes affecting skin capillaries have their reflex path over cerebral centers and are appropriately classed as psychic reactions.

When a rabbit is tied up, without being narcotized, and the ears arranged for microscopic observation of the circulation, the animal will usually, when kept warm, remain perfectly quiet over long periods if not disturbed. Any sudden noise, a slight shaking of the table or even a sudden, strong light may, however, frighten the animal and cause some muscular movements. At the same time, a very distinct vascular reaction often takes place in the ears: The capillaries and arterioles contract and the ears may become visibly paler, even to the naked eye. This contraction lasts only for a few (2 to 5) seconds and is followed by a pronounced capillary hyperemia, which subsides

gradually in the course of a minute or more. In some cases the vascular reaction is the only visible consequence of the disturbance. The afferent path for this reflex is evidently one of the specific sensory nerves (auditory or optical), the efferent path both for the contraction and for the subsequent dilatation of the vessels is through the dorsal sympathetic, since section of the sympathetic fibers abolishes the reaction in the ear concerned, while section of the sensory nerves of the ear has no influence. A light narcosis completely prevents any reaction of this type, and we have good reason, therefore, to believe, though a definite proof will require some further experimentation, that we have to do in this case with a reflex arc comprising typical brain centers and of essentially the same type as those which are responsible for the emotional vascular reactions in man.

Everybody knows that these reactions manifest themselves through distinct and sometimes quite sudden changes in the blood color of the skin. This means that they are brought about chiefly by tonus variations in the capillaries and venules. Lewis has shown by temperature measurements (p. 257) that the arterioles are also involved, since the blush is accompanied by a rise in skin temperature, while the skin which has turned pale from fear or sorrow is proverbially cold.

In analogy with the results obtained on the rabbit's ear I assume that emotional paleness is brought about, at least mainly, by an increase in the sympathetic tone of the small skin vessels, while blushing is due to a reflex relaxation of this tone.

#### *The temperature regulating mechanism.*

Another vascular reaction, which is very conspicuous in the ear of the rabbit and plays a very important rôle also in the skin of man, is worth studying

from our point of view, because it brings out very clearly the essential difference between *arteriomotor* and *capillariomotor* control of the circulation.

When a rabbit is narcotized and tied up on its back it is unable to maintain its body temperature and must be kept warm artificially. If the rectal temperature is increased above normal the ears become very hot, that is, their temperature is only  $1^{\circ}$  to  $2^{\circ}$  Centigrade below the rectal temperature. The larger arteries of the ears are strongly dilated, and the pulse in them can be both seen and felt. When the animal is cooled and the body temperature has fallen to a certain point, somewhat below the normal, depending to some extent on the depth of the narcosis and differing somewhat in different animals, the arteries of the ears suddenly contract and their temperature drops to a few degrees above the room temperature. These reactions are largely independent of the temperature to which the ears are exposed and are determined by the body temperature, which they serve to regulate, since the heat loss is obviously diminished when the temperature of the ears is nearly the same as that of the air and increased when it approaches that of the internal organs.

The enormous changes in blood flow through the ears involved in these reactions are accompanied by comparatively slight changes in the color of the ears, and microscopic observation reveals the fact that the capillaries are involved to a slight extent only and sometimes not at all. The large increase in blood flow, taking place when the body temperature rises above normal, is brought about by a dilatation of arteries and arterioles. If the number of open capillaries was very small beforehand some more will be opened up, but not to anything like the extent to which they may become opened and dilated by local stimulation. The venules and veins will, of course, become somewhat

dilated by the increased flow through them, and this brings about some reddening of the ears to naked eye inspection, but not enough to obscure the fact, which comes out very clearly by a comparison between this reaction and the local erythema, that the color of the skin depends mainly upon the state of dilatation of the cutaneous capillaries and not upon the rate of blood flow through them, while the temperature of the skin is determined primarily by the rate of blood flow, which, in its turn, depends upon the state of contraction of the arteries and arterioles.

Corresponding observations have been made on the human skin. It is well known that the effect of cold can make the skin of the hands deeply red or even blue, while at the same time the arterioles are constricted to such an extent as to reduce the temperature almost to that of the surrounding medium. Conversely Ebbecke (1923, 1) has observed in a finger, anesthetized by novocaine, how the temperature became much increased with no noticeable change in color. Ipsen (1927), who has made an extensive study of skin temperatures in man, has found that the temperature of the feet is normally low (about 30° C.). General narcosis produces regularly an abrupt rise in the temperature of the feet to about 36° with no change whatever in color. In such cases a considerable quantity of blood probably passes through arterio-venous anastomoses.

The rules to be deduced from these experiments and observations are of wide applicability and can be used as a guide to obtain an approximate estimate of the vasomotor reactions in many organs by simple ocular inspection and temperature estimates or measurements. A definite change in the blood color of an organ always implies a change in the state of contraction of its capillaries, but says nothing about the state of the arteries or the rate of blood flow.



The temperature changes can be utilized only in organs the metabolism of which is insufficient to maintain them at body temperature and which are consequently heated to a considerable extent by the flow of blood through them. In such organs a large temperature deficit means a low rate of blood flow, while a small deficit indicates a rapid flow. Stewart (1911) has elaborated an ingenious method by which the heat elimination of a hand or foot measured by a simple calorimetric device is utilized for quantitative determinations of the rate of blood flow through these limbs.

The mechanism of the vascular reactions in the skin serving for regulating the body temperature is tolerably well known. Our experiments show that the efferent nerve paths belong to the dorsal sympathetic system, since in the rabbit section of the cervical sympathetic on one side abolishes the temperature regulating changes in the corresponding ear. The afferent path is in the case of man very generally assumed to be along fibers from the temperature sense organs in the skin, but, though a reflex of this kind undoubtedly exists, its rôle as a part of the mechanism for temperature regulation has been somewhat overestimated. It can be shown experimentally on rabbits that a heating of the skin to a temperature above that of the body does not produce any considerable increase in the blood flow, unless the body temperature is at least normal, while, on the other hand, an increase of the body temperature will have this effect even when the skin is surrounded by very cold air. The stimuli sent out along the sympathetic system to regulate the body temperature must take their origin within the body, and the experiments of Barbour (1921) and others make it almost certain that a nerve center, which is directly sensitive to temperature changes and governs

the heat regulation, exists in the corpus striatum of the brain.

It is evident that the mechanism of a number of the reactions surveyed in this lecture is still largely hypothetical. I have thought it desirable, nevertheless, to discuss them at some length, because they are highly suggestive. They do show at least that the relatively simple conceptions of the peripheral nervous system which have been evolved on the basis of the neurone theory are insufficient. The new conceptions of fibrillar nerve nets, of long path axon reflexes, and of sympathetic innervation of sensory end organs may be erroneous, as they are certainly imperfect, but they point the way to a deeper insight into the nervous regulation of bodily functions to be gained by histological investigation, physiological experimentation, and clinical study.

## NOTES

<sup>1</sup> Busch, who has recently repeated these nerve sections and in other experiments also removed the sympathetic ganglia, finds that the contraction becomes completely abolished when the sympathetic fibers have degenerated. The point will require renewed experimentation.

<sup>2</sup> The vasomotor reactions in the human skin resulting from internal disease have been observed also by Zak (1922) who described a red zone (dilated minute vessels) of semilunar shape over and to both sides of manubrium sterni in cases of aortic disease. This zone he found to be spontaneously present in about one-third of the cases while in others it could be provoked by suitable stimulation of the skin. The area affected showed hyperalgesia. In some cases the reaction could also be observed within the same segment on the arms and if in such cases the part of the arm affected was stimulated by the application of hot water the redness on the breast would also become intensified after a latency of a few minutes. I suppose this latter reaction to be a case of reflex erythema and the extremely long latency I take as evidence of a slow production of H-substance from the nerve endings involved.

<sup>3</sup> Ruhmann (1927) finds that the application of heat (46° C.) to the lower part of the abdomen in man produces a reflex hyperemia of the intestine within the same segment after a latency of 4-6 seconds. The

Fig. 49 here reproduced has been obtained by inserting a laparoscope through a small incision, at some distance. The reflex is probably spinal with the efferent path through the sympathetic.

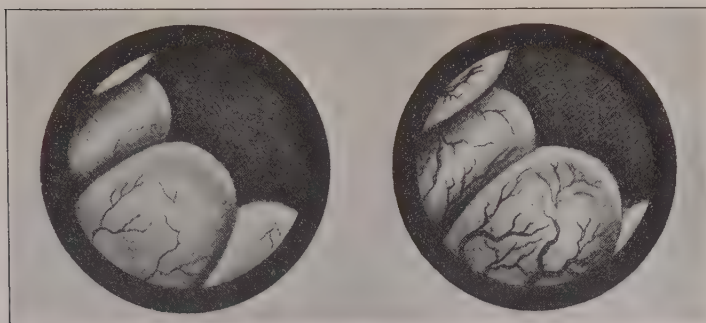


Fig. 49. Laparoscopic picture of large intestine before and 8 seconds after application of heat to the skin of the abdomen.  
After Ruhmann.

## LECTURE VIII

### THE REACTIONS OF CAPILLARIES TO DIRECT STIMULATION

IT is well known that the state of contraction or relaxation of smooth muscles can be influenced by various stimuli, and the Rouget cells forming the contractile coat of capillaries are no exception to this general rule. They can be strongly acted upon, as we have seen, through nerves; they are influenced in intricate ways by the temperature of the surrounding tissue and by temperature changes; they may relax more or less completely under the influence of a strong light. The osmotic concentration of the surrounding fluid, its hydrogen and hydroxyl ions, numerous inorganic or organic substances of a more or less well-defined constitution, which may be added to it, have a more or less definite action, and finally we have to deal with the action of substances produced within the organism itself and sent with the blood as "chemical messengers," or hormones, to regulate the state of contraction of the capillaries in various organs.

To attempt a rational classification of the factors influencing the contractile elements of the capillary wall is—at present at least—a hopeless task. One cannot help thinking with a little sigh of the conventional and delightfully simple classification of substances acting upon the general blood pressure into a "pressor" and a "depressor" group and being tempted to classify the factors acting upon the Rouget cells as "constrictor" and "dilator," respectively, but such a

classification would be nearly as wrong in principle, and might prove quite as harmful in its application, as the pressor-depressor classification itself, because it might put together in one class factors which differed in kind of action and separate others which differed only in degree. We are confronted further with the difficulty which also may sometimes upset the most beautiful pressor-depressor classification, namely, that one and the same substance or physical factor may act, according to concentration and circumstances, sometimes as a constrictor and sometimes as a dilator.

As is generally the case in physiological research, we have a double purpose in studying the reactions of capillaries to physical and chemical stimuli: we want to find out the mechanism (in the broadest sense of that term) of every single reaction studied, and we want to find out the meaning, the part played by the reactions in the delicate regulations by which the organism and the organs are adapted to the ever changing environmental conditions. Neither of these purposes must be lost sight of, and in many cases they are, of course, so closely connected that they cannot be separated; but generally, I think, it is conducive to good economy to keep them as separate as possible, both in research work and in the presentation of its results.

#### *Direct and indirect capillary reactions.*

When a substance acts upon a capillary field and brings about, say, dilatation, we can, according to present physiological notions, distinguish between the following mechanisms, any of which may possibly be involved in the reaction, either alone or in combination with one or more of the others.

We may have either a local action or we may have an action at a distance through nerves or other propa-



gating tissue. The first of these mechanisms involves several quite distinct possibilities. The stimulating substance may act upon the contractile cells themselves, it may act upon their myoneural junctions, the somewhat hypothetic elements interposed between the nerve fibers and the muscle cells and usually acted upon through the nerve fibers, or it may, finally, act upon the tissues generally, altering the "environment" of the capillaries and their contractile cells.

Thanks to the work of Thomas Lewis and his collaborators we can now in many cases definitely distinguish between reactions taking place locally, through a *direct* action on elements in the capillary wall, and *indirect* reactions, due to certain substances liberated from tissue cells in their reaction to stimulation.

In the local reactions, whether direct or indirect, spreading of the effect beyond the area stimulated is often observed. Slow spreading (of the order of 1 mm. per minute or less) is normally due to diffusion of the acting substance itself, but reactions taking place, usually after a short period of latency, over a larger area or at points more or less distant from the one stimulated must be propagated through some conducting structure.

It is possible that the Rouget cells on some capillaries may form a syncytium in which a contraction may be propagated from one cell to another, but the observations so far made have failed to reveal any direct connections between the cells. A propagation of this kind would reveal itself as a contraction or relaxation progressing *slowly* along a capillary from a single point. In the frog we have never seen this form of contraction, but there are a few observations on the skin of man (Ebbecke, 1917) which may possibly be explained in this way. Generally, however, there can be

no doubt that in reactions at a distance we have to do with a propagation along nerve fibers. Here again we have to distinguish, whenever possible, between the two different mechanisms already described: we may have to do either with an axon reflex or with a true reflex.

Whenever the reaction to a stimulus is essentially the same outside as inside an area stimulated, it will be natural to conclude that the stimulus acts primarily on nerves, and in all cases where no spreading whatever occurs we must assume an action on excitable tissue without the intervention of nerves, but in many cases we have a combined action on blood vessels and nerve endings simultaneously, and it is often extremely difficult to assess the part played by each in the resulting reaction.

We shall deal in this lecture with

#### *Reactions of capillaries to direct stimulation.*

The reactions put together under this head are those in which, *according to our present knowledge*, the stimulus acts directly on elements of the wall of the small vessels without the intervention of nerves or tissue cells. It is necessary to emphasize the words, "according to our present knowledge," because in many cases it is not possible to state definitely that neither the local nervous elements nor products liberated from tissue cells play any part in the reactions.

#### *Direct reactions to mechanical stimulation.*

When the end of a flat ruler (2-3 cm. broad, smooth, and with edges just rounded) is drawn steadily, but not roughly, across the human skin the area covered by the stroke becomes after 15-20 seconds distinctly paler than the surroundings. Miss Carrier (1922) showed by microscopic observation that the blanching

is due to contraction of the skin capillaries and venules, and Heimberger (1925) has shown further that a sharply localized weak mechanical stimulus will generally result in a contraction of a length of capillary corresponding probably to one or two Rouget cells. Miss Carrier and Heimberger agree that the contraction on mechanical stimulation is preceded by a dilatation comprising the capillary and, since the flow is increased, the arteriole supplying it, but not the venule. This initial dilatation is too slight to be seen macroscopically and is probably due to an axon reflex which will require further study.

Lewis (Cotton, Slade, and L., 1917) found that the white reaction is independent of the blood flow and can be obtained equally well on an arm in which the arterial supply has been cut off for 10 minutes, when the arm is distinctly cyanotic (see Fig. 50). Ebbecke (1917) found that the reaction could be produced after damage to the corresponding peripheral nerves, and even in cases where these had degenerated, and that local anesthesia with novocaine did not prevent its occurrence, and these observations have been confirmed (Carrier, Lewis).

All these observations point to the conclusion that the Rouget cells on the capillaries and venules of the human skin react by contraction when stimulated mechanically. Lewis describes experiments which make it probable that the most effective stimulus is stretching of the vessel walls, and it is natural to conclude that we have to do with a direct reaction of the Rouget cells, similar to that shown by the arterial musculature as a response to stretching. The experiments of Heimberger give strong support to such a conclusion. The force exerted in the contraction is very considerable (Lewis, p. 32).

I have been unable to obtain the white reaction to

mechanical stimuli macroscopically or microscopically on the ears of rabbits, but it has been observed by Ebbecke (1917) on internal organs, especially on the surface of the kidney, and Florey (1925) finds that some capillaries leaving the arterioles of the pia mater

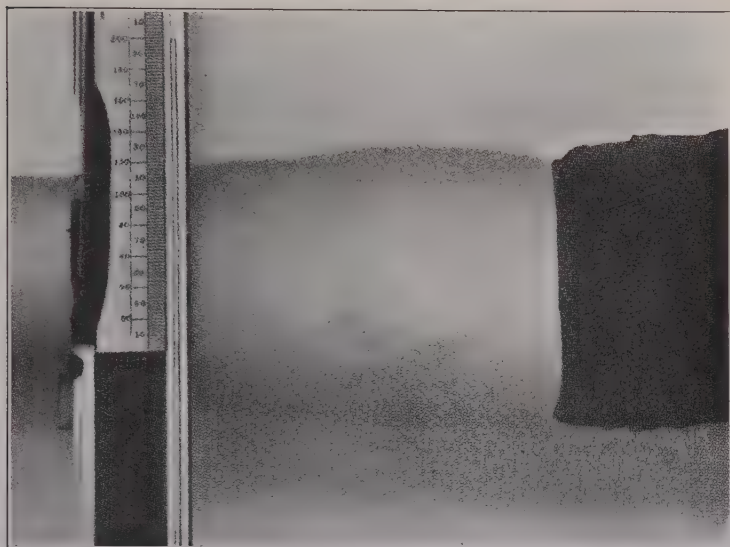


Fig. 50. White reaction with circulation stopped. In a subject whose systolic blood pressure was 110 mm. Hg., the circulation to the limb was arrested by throwing a pressure of 200 mm. Hg. into the pneumatic arm-let. Two minutes later the arm was stroked transversely in two places with a flat ruler. The white reaction shown was photographed four minutes after occlusion of the vessels. After Lewis.

for the cerebral cortex contract to mechanical stimuli, but that all the capillaries do not so react. In the frog's tongue and also in the skin the normal reaction to weak mechanical stimulation is relaxation, while stronger stimuli may in the skin produce contraction. These reactions in the frog are probably brought about

through the intervention of nerves, but even so it is certainly unsafe to assume that the normal response of all Rouget cells to mechanical tension is a contraction which may, however, be obscured by simultaneous indirect and nervous reactions.

*Direct reactions to electrical stimulation.*

Liebermann (1921) observed on frogs' arteries that passing of a constant current for some seconds through unpolarizable electrodes would produce sharply localized constriction at the kathode and dilatation at the



Fig. 51. Successive alterations in form of corpuscle passing contracted point in capillary of bat's wing. After Ni.

anode, but could not find any corresponding reaction on capillaries, while Ni (1922) obtained very sharply localized constrictions on capillaries in frogs' webs, fins of fishes, wings of bats, and fingers of human subjects by applying series of make and break shocks, from either an induction coil or a battery. Tetanizing currents were frequently ineffective. Fig. 51 taken from Ni's paper shows the change of form of two corpuscles passing through the constriction in a capillary in the wing of a bat. The length of the constricted part seems to be no more than  $2.4\mu$ . It is inconceivable that such a constriction could be brought about indirectly. The stimuli must act either on a few fibrils in a single Rouget cell or on single endothelial cells. Heimberger (III, 1926) finds on the nail capillaries of man that the constant current anode will bring the Rouget cells to contraction.



*Direct responses of capillaries to heat and cold.*

According to Lewis (p. 149) the minute vessels of the skin of man respond to low temperature ( $10^{\circ}$ - $20^{\circ}$  C.) by relaxation, and the well-known redness of the hands from handling snow is due to this reaction. The response is strictly limited to the area cooled and is not influenced by section or degeneration of the nerves supplying that area. The recovery, taking place at higher temperature, is not influenced by cutting off the blood supply, but takes place more rapidly at  $32^{\circ}$ , which is about the normal skin temperature, than at  $25^{\circ}$ . Lewis believes that these decreases and increases in tone by moderate changes in temperature are the only "truly direct" influences of temperature that we are now entitled to postulate—all other reactions, and especially the relaxation to heat, being indirect.

It is incontestable that the skin capillaries and venules become relaxed by cooling, but it has not been proved that even this response is strictly direct. The initial response to cooling is certainly contraction of all the minute vessels of the human skin (Breslauer, 1919; Bruns and König, 1920; Carrier, 1922; Weil, 1924), which contraction is obtained also in cases where the nerves have degenerated, as Breslauer has definitely shown. The arterioles keep up the contraction, but the capillaries and venules relax (are "paralyzed by cold") after a variable period of one to a few minutes, depending upon the temperature.<sup>1</sup> Contraction as a response to heating above the normal skin temperature has not been observed.

In the experiments of Natus (1910) the irrigation of the rabbit's pancreas with saline of  $22^{\circ}$  C. gave as the only visible change a slowing of the blood stream, due perhaps to the increase in viscosity of the plasma, perhaps also to some contraction of arteries. At lower temperatures ( $7^{\circ}$  to  $5^{\circ}$ ) there is definite evidence of a

considerable contraction of arteries, while the emptying of capillaries observed, and reported as contraction, may possibly be due to plasma skimming. In the frog's tongue I have found (1920) that the response to cooling to about 2° C. is relaxation of capillaries without any initial contraction. Heating to a temperature well above that of the room produces relaxation and hyperemia in the normal tongue, but fails to elicit any visible response after cocaineization.

#### *Reactions to hydrogen ions.*

A large number of investigations, among which special reference should be made to the famous experiments of Chauveau and Kaufmann (1887) on the levator labii muscle of the horse and to the beautiful researches of Barcroft (1908, 1915) on the submaxillary gland, have shown that the flow of blood to active organs is increased, and that this *functional hyperemia* is brought about by some reaction on the part of the active tissue itself. It is generally believed that this reaction is simply the increased formation of acid metabolites, especially carbonic acid, which is inseparably bound up with increased activity, but I am afraid it must be admitted that the foundations of this latter belief are not very secure. It has been shown repeatedly that the addition of very dilute acids to perfusion fluids will increase the volume perfused at a given pressure, or, in other words, produce a decrease in the resistance, which must be due to arterial dilatation, but Fleisch (1921) has shown that in almost all the experiments made the hydrogen ion concentrations employed have been many times higher than they can ever occur in living tissue.

Let me recall to you that in normal arterial blood there is 1 gram equivalent of hydrogen ions in 22 million liters of blood, that is, the hydrogen ion concen-

tration is  $1:10^{7.35}$  normal, or, as it is generally expressed, the  $p_H$  of the blood is 7.35. The normal  $p_H$  of mixed venous blood is 7.3. The acidity of the tissues themselves is perhaps a little higher and with maximum activity it may conceivably rise to  $p_H = 7$ . The  $p_H$  of the solutions that have been generally used to demonstrate the dilator effect of acids in perfusion experiments has been in the neighborhood of five or even four; that is, they have been from a hundred to several thousand times more acid than the blood can possibly become. Fleisch himself has made a very beautiful series of experiments with perfusion fluids, which were buffered by the addition of phosphates so as to maintain a definite  $p_H$  which could be adjusted to any desired figure, and he has shown that an increase in hydrogen ion concentration even from  $p_H = 7.6$  to  $p_H = 7.5$  will produce a distinct increase in the volume perfused. The increases obtained by Fleisch with hydrogen ion concentrations within physiological limits are certainly too small, however, to account for the increase in blood flow through active organs, and it must be argued further that his perfusion fluids contained too little oxygen to supply the needs of the tissues. Lack of oxygen is, as we shall see, a very serious complicating factor, and I find it impossible to accept as binding the evidence at present available for the view that the increased supply of blood to active organs is brought about exclusively or even mainly by the vasodilator action of *acid* metabolites.<sup>2</sup>

From the point of view of these lectures we are interested not so much in the absolute increase in blood flow through active organs as in the simultaneous opening up and dilatation of capillaries, which probably take place in all organs during activity and which have been demonstrated most clearly in the case of muscles. We shall examine, therefore, the available evidence re-

garding acids, as substances which bring about capillary dilatation. We shall have to consider first a few experiments in which acid solutions have been brought in contact with the tissues from outside. These will require some introductory words of explanation.

Any acid or any substance having an acid reaction will, when brought in contact with living tissue, bring about a wandering, in all probability by simple diffusion, of hydrogen ions into the tissue. These hydrogen ions will increase in turn the hydrogen ion concentration of the tissue, but to what extent that will take place cannot be predicted with any certainty, because the living tissues and the blood contain the so-called "buffer" substances (notably bicarbonates) and resist by chemical combination any increase in their hydrogen ion concentration. All that can be said, therefore, is that within the tissue, so long as it is living, the increase will probably be very small compared with the hydrogen ion concentration of the acid solution applied. It is important to bear this fact in mind, when the following experiments are considered.

When a small drop of 1 per cent acetic acid is applied to the ventral surface of the spread tongue of a frog, dilatation of both arteries and capillaries takes place, and the reaction spreads at once to a distance of a couple of millimeters beyond the area directly affected. When the surface of the tongue is cocaineized, or when the lingual nerves have been cut, the reaction is still present, but it is much diminished in intensity, especially with regard to the capillaries, and it does not spread. It follows from these observations that a certain, so far undefined, but probably considerable, increase in the hydrogen ion concentration will affect the sensory nerve endings of the tongue and also directly the smooth muscle elements of the arterioles and to a less extent of the capillaries as well.

In another experiment—performed for me by Dr. Harrop—buffer mixtures of definite hydrogen ion concentrations were made up and applied to the ventral surface of the tongue in reaction basins, which are paraffined brass rings of 3 to 4 mm. inside, and 6 to 8 mm. outside, diameter, put on to the tissue under investigation and filled with the fluid, the effect of which is to be studied. The buffers used were Sörensen's mixtures of sodium citrate with  $\frac{N}{10}$  hydrochloric acid.

To our surprise we had to use very acid mixtures to obtain any dilator effects on the capillaries. A mixture of 5 volumes citrate with 5 volumes HCl having a  $p_H = 3.65$  had no effect, and even for the next mixture, 4 citrate + 6 HCl with a  $p_H$  of 2.96, the result was very doubtful, while 3 citrate + 7 HCl,  $p_H = 1.94$ , gave a definite, but slight, dilatation after a short latency. Pure  $\frac{N}{10}$  HCl,  $p_H = 1$ , gave a well-marked dilatation. In all these experiments the actual increase in acidity within the tissue may have been quite small.

A definite change in the hydrogen ion concentration of the tissue can be brought about by changes in its carbon dioxide tension. We have at present no definite reason to believe that  $CO_2$  acts in any other way than by forming with water a very weak acid, and, in any case, stronger acids, which are formed in or brought into the tissue, will react in the first instance with the bicarbonates present and thereby raise the  $CO_2$  tension. The spread tongue of a frog was exposed to  $CO_2$  by means of the apparatus shown in Fig. 52—a slight modification of Roy and Brown's apparatus for measuring capillary blood pressure, mentioned in my second lecture. The gas or gas mixture is led into the lower chamber, the top of which is covered with a thin peritoneal membrane, thence through the tubing to the



upper chamber and from this to a tube dipping in water. The spread tongue is inserted between the two chambers and the slight gas pressure maintained is sufficient to make the system gas tight. When a current of pure  $\text{CO}_2$  gas is led through this apparatus it will produce, after about one minute, a considerable hyperemia, with dilatation of both arteries and capillaries and a rapid current of blood. A mixture of 10 per cent  $\text{CO}_2$  in air produced in one experiment after a couple of minutes a distinct increase in the circula-

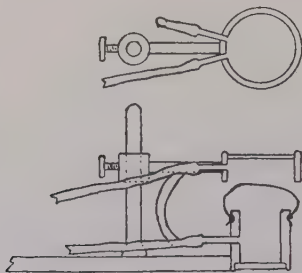


Fig. 52. Apparatus for application of gases to capillaries under the microscope.

tion through a few capillaries selected for observation, but the general increase over the whole field was too slight to be definitely ascertained, and no widening of capillaries could be made out. In these experiments the  $\text{CO}_2$  tension of the tissue corresponded, at least very nearly, to the  $\text{CO}_2$  percentage of the gas mixture. The  $\text{CO}_2$  diffuses very rapidly through the tissues, as will be shown in some detail in a following lecture (XII). It will enter into chemical combination to a certain extent at first, but saturation will be reached very soon, and, though some  $\text{CO}_2$  is undoubtedly carried off by the blood, this is compensated by

the constant formation of  $\text{CO}_2$  in the tissue. The actual hydrogen ion concentration reached in such an experiment can be made out with considerable accuracy by saturating frog's blood with  $\text{CO}_2$  at the same pressure and determining its  $p_{\text{H}}$  by a suitable method. We found by a colorimetric method that saturation with 10 per cent  $\text{CO}_2$  would lower the  $p_{\text{H}}$  of frog's blood from 7.5 to 7.1.

For practical purposes it is sufficient, however, to consider the  $\text{CO}_2$  tensions. Normal frog's blood possesses a  $\text{CO}_2$  tension between 1 and 2 per cent of an atmosphere (Krogh, 1904); it will be in diffusion equilibrium as regards  $\text{CO}_2$  with an atmosphere containing between 1 and 2 per cent  $\text{CO}_2$ . A  $\text{CO}_2$  tension of 10 per cent, therefore, means a degree of acidity which probably never occurs in the normal frog, and it follows that, though the vessels, and especially the arteries, of the frog's tongue are dilated under the influence of increased hydrogen ion concentration, this reaction cannot play any considerable rôle in the normal regulation of the blood supply and probably plays no part whatever as a regulator of the capillary circulation.

We have now to consider some experiments and observations on the rabbit's ear made by Rehberg and myself. They were undertaken for another primary purpose, to which I shall have to return later, but incidentally they can be used to give some information on the problem under consideration.

In the first of these experiments the rabbit was breathing through a tracheal cannula, while the depilated transparent ears were watched both macroscopically and microscopically by transmitted light. When the "dead space" of the lungs was increased by adding a tube of 15 cc. to the tracheal cannula, a very pronounced hyperemia of the ears, comprising capil-

laries as well as arteries, set in after about two minutes. At the same time the blood became visibly cyanotic. When the extra dead space was removed the hyperemia disappeared in the course of one-half to one minute. By increasing the dead space to 35 cc., the effects could be intensified and brought about more quickly. The cyanosis in such an experiment became very pronounced. These experiments show that an increased vensity of the blood brings about both arterial and capillary dilatation, but they leave it undecided whether the dilatation is central or peripheral in origin, and whether it is due to an increase in  $\text{CO}_2$  tension or to lack of oxygen, or both.

In a further experiment a rabbit was made to breathe air with 10 per cent  $\text{CO}_2$  from a spirometer. The  $\text{CO}_2$  percentage of the expired air rose to 11.47 per cent, but, in spite of this considerable increase in the hydrogen ion concentration, no dilatation of capillaries or arteries could be detected in the ears. When, however, the rabbit was given about 14 per cent  $\text{CO}_2$  to breathe, a definite hyperemia could be made out. This corresponds to a decrease in the  $p_{\text{H}}$  of about 0.4.

*Certain substances produce capillary contraction.*

Sandor reports (1926) that caffeine (in a concentration of 1-100,000) applied to the vessels of the frog's brain will produce capillary constriction, and Barksdale (1925) has found that guanidine derivatives (dimethyl-guanidine sulphate) injected intraperitoneally in frogs, pigeons, and rabbits in quantities of 1 to 10 mg. will produce a very pronounced but short-lived contraction of capillaries in the web, brain, and external ear. Referring to the observation of Major and Stephenson (1924) that guanidine is normally excreted by human subjects in quantities of about 100 mg. per day, while the excretion is markedly diminished in

cases showing hypertension, Barksdale believes that the capillary constriction observed may be the cause of the hypertension. This belief would not, however, be warranted by the evidence, even if the results of Major and Stephenson were beyond doubt. Neither in the case of caffeine nor in that of guanidine is it possible at present to say anything about the mechanism of the reaction.

The case is different with regard to adrenaline, at least in some of the cases, where this substance acts upon capillaries.

The action of adrenaline upon capillaries is, perhaps, even more puzzling than its action on arteries. Until recently it came pretty near to being an axiom in physiology that adrenaline was strictly sympathicomimetic and could be relied on to have the same effect on any tissue as that produced by a stimulation of the corresponding sympathetic fibers. Since a number of capillaries have been shown to be innervated through the sympathetic, the stimulation of which causes prompt contraction, this proposition would demand that the capillaries should respond by contraction when a suitably dilute solution of adrenaline was applied to their walls. Certain capillaries do so respond, but others undoubtedly do not. Since the reactions of capillaries to adrenaline are bound up more or less closely with those of arteries, it will be necessary in the following discussion to deal also with certain aspects of the response of arteries to adrenaline.

In the tongue of the green frog (*R. esculenta*) I have seen that the capillaries invariably respond to the application of 0.1 per cent adrenaline, in small or large drops or in reagent basins, by distinct and, as a rule, very pronounced dilatation. Most of the smaller arteries and arterioles also dilate, while the larger arteries remain unaffected. A few small arteries have

been observed to contract for a brief period and in one of these cases a simultaneous dilatation of the corresponding capillaries was distinctly seen.

In the tongue of the brown frog (*R. platyrrhina*) I have found numerous arteries susceptible to the action of adrenaline and they contract strongly, but several, and especially the largest, arteries were not affected and the capillaries in my experiments dilated as in *R. esculenta*.

The results obtained by Killian (1925) and objectively demonstrated by beautiful photographs are very different. Killian studied the vessels of the tongue in the brown frog (*R. platyrrhina*) and applied adrenaline locally, in concentrations from 1-1,000 to 1-1,000,000, and also generally by injection in a lymph space; he invariably obtained a contraction of capillaries as well as of arterioles and arteries. The action on the arterioles and arterial capillaries was very pronounced and they usually contracted to obliteration after the application of solutions above 1-100,000. The action observed on venous capillaries and veins was late and doubtful. I am unable to explain the discrepancy between Killian's observations and my own, and the matter evidently requires further experimentation.

In the internal organs of the frog (*R. esculenta*: stomach, intestine, bladder) numerous arteries and arterioles are refractory toward adrenaline, and capillaries have not been observed to respond at all.

In the muscles, on the other hand, all the arteries and arterioles so far tested have responded very promptly to the application of even the smallest drops of adrenaline, while *careful* observation shows the capillaries to remain unaffected in spite of the fact that they can be brought to contraction by stimulation of the sympathetic. It is necessary to emphasize the careful observation, because in this test plasma skimming



is particularly apt to occur and will give the appearance of capillary contraction, which is the more difficult to guard against, in that the walls of the capillaries are generally very difficult to see between the muscle fibers.

In the skin and web of the brown frog the reactions to adrenaline have been observed more closely and a very definite discrepancy between them and the responses to sympathetic stimulation has been made out. The capillaries are all refractory toward adrenaline, though prompt contractions can be evoked by stimulation of the sympathetic. Most of the arterioles and small arteries are also refractory, though the larger arterial branches (above about 0.1 mm. external diameter in a dilated condition) contract readily. Usually the limit between the refractory and responsive portions of arteries is quite sharp, as can be demonstrated by the following experiment.

Small drops ( $0.001 \text{ mm.}^3$ ) of 0.1 per cent adrenaline are placed on the skin or web just over the superficial branches of arteries. When such a drop is placed over an arteriole it shows no constrictor effect. By following up the artery, putting drop after drop over its course, a point is finally reached where the constrictor reaction begins. When the drops are placed a short distance apart and half a minute or more allowed to elapse between the single tests, it is often observed that the artery begins to contract at a certain distance proximally from the last drop, and the contraction spreads slowly in a proximal direction, while the artery just below the last drop remains open. By repetitions of the experiment the limit of the adrenaline constriction remains the same on the same artery, and we have convinced ourselves in special tests that the arterial branches which are refractory to adrenaline respond just as promptly to sympathetic stimulation

as do the others. On the film illustrating these lectures it is shown how the proximal part of a web artery contracts to adrenaline while the distal end remains wide.

W. Jacobj (1920, 1921) has found that dilute adrenaline solutions (from 0.03 per cent downward) are inactive when applied to the web of the frog, but that a previous treatment with 5 per cent veronal sodium or 1 to 8 per cent sodium carbonate for some minutes will render even extremely dilute adrenaline solutions (down to one in a million) effective. Jacobj ascribes this effect of alkaline solutions to an increased permeability of the frog's epidermis and shows that the absorption of other substances (strychnine) is greatly facilitated by the treatment with alkali. I have confirmed the observation of W. Jacobj, but I find that those arteries which were refractory before also remain so after the treatment. They are not, therefore, as might be supposed, merely less sensitive to adrenaline, but are insensitive.

In the case of mammals Hooker (1920) reports observations of contractions of capillaries and venules in the cat's ear after intravenous injection of 0.06 mg. adrenaline, and in connection with these observations reference must be made to the puzzling experiments of Dale and Richards, mentioned in some detail in a former lecture. In these experiments it was demonstrated that a minute dose (0.004 mg.) of adrenaline, injected intravenously in a cat, produces an evanescent vasodilator reaction, which must probably be located in the skin capillaries, while a larger dose gives the usual vasoconstrictor response, which, as we now may infer from Hooker's experiments, involves the capillaries as well as the arteries.

Several statements, notably by Ricker and Regendanz (1921), which seem to imply a constrictor effect of adrenaline upon the capillaries of the rabbit and other

animals, are not sufficiently clear to be accepted as evidence, the more so as the sources of error have not been recognized. In the rabbit's ear the effects of local application have been tested microscopically by Rehberg and myself with entirely negative results, as far as capillaries and venules were concerned. Quite recently (1928) Hartman, Evans, and Walker have published observations on the muscle capillaries of cats and rabbits to show that adrenaline in small doses causes dilatation and opening up of muscle capillaries with increase in flow. Larger doses while still dilating capillaries constrict the arterioles.

In the case of man Cotton, Slade, and Lewis (1917) have found that the introduction of adrenaline beneath the skin will produce, after a latency of fifteen seconds to one minute, an intense blanching. This might be due to constriction of arterioles and washing out of the capillaries with plasma, but, when they find that the same blanching can be produced by adrenaline five or more minutes after the circulation has been brought to a standstill by means of an occluding armlet, they rightly conclude that the capillaries and venules themselves must contract to be emptied under these circumstances.

Miss Carrier (1922) and Heimberger (1925) have shown that quite short lengths of the capillary loops can be brought to contraction by the introduction into the tissue of a minute drop of adrenaline, and it is natural, therefore, to assume a direct action on the Rouget cells, though in other cases neighboring capillaries, venules, and arterioles contract also and simultaneously. Heimberger concludes that in these cases nervous elements are involved, but I think it possible that the adrenaline may have been injected into a perivascular lymph space and thereby rapidly distributed. Miss Carrier has studied the local action of very dilute

solutions of adrenaline 1-100,000 and 1-1,000,000 in the hope of observing dilatations like those inferred by Dale and Richards, but has failed to observe anything but contraction. The solution of 1-1,000,000 could not be distinguished from saline.

Lewis (1923) has measured the force exerted by capillaries and venules contracting under adrenaline by raising the venous pressure to measured heights, and he finds that the vessels are able to withstand pressures of 80 or even 100 mm. Hg. without dilating. When, however, the pressure is put on before adrenaline is administered they are unable to contract against pressures above 40 or at most 60 mm. Hg. If adrenaline is punctured into the foot of a person standing at rest blanching will generally not occur, because the pressure is too high (see p. 302).

In certain conditions and especially in inflamed tissue the vessels become unresponsive to adrenaline, and the hormone may even cause dilatation as Ricker and Regendanz (1921) have found in experimental inflammation of the rabbit's peritoneum brought about by chemical stimuli. Lewis finds that the minute vessels of the human skin, when injured so as to become permeable to plasma proteins, are for a time unresponsive to adrenaline. Even capillaries and venules which are overdistended, but not abnormally permeable, may show this unresponsiveness about the mechanism of which only guesses can be made.

## NOTES

<sup>1</sup> The responses of human skin capillaries to moderate changes in temperature cannot at present be satisfactorily analyzed. Weil (1924) has found as the normal response to a piece of ice placed for 10-40 seconds on the back of the hand that the capillaries and venules remained empty (contracted) for  $\frac{1}{2}$ -1 minute after the ice had been removed. Thereafter, relaxation took place both in arterioles, capillaries, and venules, and a "reactive hyperemia" lasting 1-2 minutes was normally observed. If the

subjects had a warm bath (38° C.) the reactive hyperemia to the ice test became much diminished for a period of 20 minutes to 1 hour afterward. Cold baths of short duration (5 minutes at 20° C.) also reduced the reaction to the ice test for some time afterward.

<sup>2</sup> Recently (1926) Hemingway and McDowall have made perfusion experiments on the hind limbs of cats and confirm the fact that a  $p_H$  of 7.4 to 7.3 is essential for the maintenance of normal tone in the vessels regulating the flow of blood—which they take to be capillaries.



## LECTURE IX

### THE REACTIONS OF CAPILLARIES TO DIRECT STIMULATION (continued)

#### *A pituitary hormone.<sup>1</sup>*

EVIDENCE has been accumulated showing that a pituitary principle acts as a constricting agent upon several different capillaries and perhaps upon capillaries generally and that this principle normally circulates in the blood.

The beginning was made by Rehberg who extirpated the pituitary on frogs (*R. temporaria*) and studied the effect on the capillary circulation in the skin and web.

For the first few hours after the operation there is no apparent change in the circulation, but thereafter the capillaries in the skin and web begin to dilate, and after twenty-four hours they are usually strongly dilated. At the same time another change takes place, which has nothing to do with the circulation, but which has proved very helpful in the study of pituitary function. The frog (*R. temporaria*) becomes much lighter in color, in fact, quite pale. The microscope reveals the fact that this change is due to the black pigment cells in the skin, which are usually expanded and greatly ramified, but which, in the operated animals, contract into small balls, intensely black, but occupying only a minute fraction of the skin area.

The color of a frog, in which the whole of the pituitary gland has been removed, remains pale, but the state of the capillaries undergoes some remarkable

changes. After a variable period, generally one or two weeks, the capillaries regain their ability to contract, but the cutaneous circulation is now characterized by its want of equilibrium, just as may be the case in a definite area after section of the corresponding nerves. States of extreme contraction may change abruptly into pronounced or even maximal dilatation, and a circulation which can be accepted as normal is observed only occasionally for short periods. It might be supposed that at this stage cutting of the sciatic would abolish completely the control of the animal over the circulation in the web. This does not seem to be the case, however, but the point has not as yet been sufficiently investigated. The frogs from which the hypophysis has been removed usually survive the operation only for two to four weeks.

It is well known that the hypophysis is not a single organ with one well-defined function, but consists of several portions which differ greatly in their histological structure. Distinction is usually made between a glandular portion (often termed the *pars anterior*), a nervous portion (*pars posterior*), and an intermediate portion. The terms *anterior* and *posterior* are rather unfortunate, because in the frog the hindmost part is the one having a glandular structure and corresponding, therefore, to the *pars anterior* in mammals.<sup>2</sup>

While the complete removal of the hypophysis of the frog requires some operative dexterity, the removal of the glandular portion alone is quite easy, and this operation has been performed repeatedly. We find that the initial effects on the cutaneous circulation and pigmentation are the same as those resulting from removal of the whole gland, but in a week or less the color begins to darken and a normal capillary circulation is restored. The animals are able to survive this operation for an indefinite period. We conclude,

therefore, that the glandular portion is not the one responsible for the production of the capillary hormone, though its removal disturbs for a time the function of the really active tissue, which must be located either in the nervous or in the intermediate portion.

*The effects of pituitary extracts.*

If a hormone which increases the contractile tonus of capillaries is normally produced by the pars nervosa or intermedia of the pituitary gland we must expect to find it in the pituitary extracts which are commercially obtainable. We have tested thoroughly the extract prepared by Parke, Davis & Co., from the "posterior" portion of the pituitary and sold under the name of pituitrine. This extract is, in reality, prepared from the nervous and intermediate portions of the hypophysis of cows, and 1 cc. of the extract is stated to correspond to 0.2 g. of the fresh pituitary substance.

Injection into the web of a frog of a minute drop of this extract brings about the contraction of both arteries and capillaries, while the veins remain unaffected. When a highly diluted extract is injected the action on the arteries may be absent, but the capillaries are still strongly affected and may contract even to obliteration. The intact epidermis is impermeable to the active substance, but when the web is treated for a few minutes with 5 to 10 per cent veronal sodium it becomes permeable, and the subsequent application of pituitary extract diluted to 1/100 causes definite contraction of the capillaries, a number of which may even become closed.

*Perfusion experiments with pituitary extracts.*

It is evident that the pituitary extract has a specific action upon the capillaries in the frog's skin, but if it

is to be accepted as containing a hormone normally present in the blood, it must be shown to be still active in much smaller concentrations. To investigate this point we (Rehberg and myself) made perfusion experiments on the hind legs of brown frogs. Our first perfusion fluid was made up from Ringer solution, to which was added 0.1 per cent glucose,  $1/3$  volume of washed red corpuscles from the ox and  $1/10000$  commercial pituitrine. It had the effect of promptly stopping the circulation by causing complete contraction of both arteries and capillaries.

The following experiments with weaker concentrations of pituitrine showed that the addition to the Ringer of 1 in 50,000 to 1 in a million parts pituitrine generally had the desired effect of maintaining the tonus of the capillaries without causing complete contraction and without having any visible influence upon the arteries. The addition of 1 part pituitrine to 5 million parts perfusion fluid had no perceptible influence in the few cases in which it was tried. I shall reproduce one protocol in some detail as an example.

Femoral artery clamped 3<sup>50</sup>.

Perfusion with 3 per cent gum Ringer + pituitrine 1:1000000 begun 3<sup>52</sup>.

We found it advisable always to begin the perfusion with a solution free from corpuscles to wash out the frog's own blood and make sure by the disappearance of all corpuscles that no blood got in by collateral circulation from the frog.

Perfusion with the same fluid +  $1/3$  vol. washed ox corpuscles begun 4<sup>00</sup>. The circulation is, on the whole, in a good condition. Capillary network rather dilated. Stasis in a few capillaries.

4<sup>13</sup> Capillaries become distinctly narrower.

4<sup>15</sup> A large number of capillaries very narrow.

The transient contraction of capillaries at 4<sup>15</sup> may very probably have been due to innervation. We found it advisable in later experiments to cut the sciatic some minutes before beginning a perfusion experiment.

4<sup>25</sup> Capillary network about normal.

4<sup>28</sup> Changed to a perfusion fluid free from pituitrine.

4<sup>35</sup> Some dilatation of capillaries. Sciatic nerve cut.

5<sup>23</sup> Capillaries much dilated. Stasis developing in several places.

5<sup>25</sup> Changed to gum Ringer + pituitrine 1:1000000.

5<sup>26</sup> Changed to gum Ringer + corpuscles + pituitrine.

The capillary network now began to contract and the following measurements were made of the diameters of seven capillaries selected for the purpose. The measurements are in arbitrary scale divisions.

5 <sup>32</sup>	1.5	1.8	1.5	1.0	1.2	1.0	1.7	Total	9.7
5 <sup>41</sup>	1.1	1.0	1.7	0.8	1.3	1.2	1.6	"	8.7
5 <sup>47</sup>	1.1	1.0	1.3	0.8	0.5	0.8	1.0	"	6.5
5 <sup>55</sup>	1.0	1.1	1.5	0.6	0.4	0.7	0.8	"	6.1

7<sup>00</sup> Stasis has developed in a large number of capillaries and many small bleedings have occurred from these (the web had been allowed to dry up somewhat). In those capillaries which are still open the circulation is good, and these capillaries are, on the whole, narrow, some of them very narrow.

The problem concerning the causation and prevention of stasis in the capillaries is a complicated one, and its theoretical aspects can be more conveniently dealt with in a later chapter, but certain facts must be pointed out here, because they are of great practical importance in testing the tonus of capillaries by means of perfusion experiments.

The development of stasis depends upon the rate at which fluid leaves the capillaries through the endothelial wall. If this rate is so rapid that the corpuscle emulsion becomes concentrated beyond a certain point the corpuscles stick together and block the passage. This increases the pressure and a vicious circle is thus set up, rapidly leading up to the capillaries being filled with a densely packed mass of corpuscles.



When the perfusion fluid does not contain colloids filtration takes place, and when the capillaries have become dilated beyond a certain point the rate of filtration is increased and stasis often develops with astonishing rapidity.

When the perfusion fluid contains a suitable colloid, such as 3 per cent gum acacia, the filtration of water through the normal capillary wall is practically inhibited, and stasis develops only when the capillary wall becomes permeable to the gum molecules. The causes bringing about this change are not understood in their entirety, but one important condition may certainly be a blood pressure in excess of the normal. It appears that the capillaries in the web of a frog are able to withstand an abnormally high pressure for some time, but finally they give way and become relaxed to such an extent that the colloidal particles can pass out. The presence of a substance like pituitrine counteracts the tendency to relax under an abnormally high pressure, but seems to be able only to delay the final relaxation. The influence of pituitrine is brought out very clearly in perfusion experiments without gum, where stasis occurs in the absence of pituitrine as soon as the capillaries are relaxed. With the addition of gum and a low pressure, that is, with the conditions as nearly physiological as possible, the circulation can be maintained sometimes for hours without the addition of pituitrine, but after the first half hour or so nearly all the capillaries become strongly dilated and remain so, while with pituitrine practically all the capillaries remain narrow.

As an example of this typical difference, I give the following protocol of an experiment in which both legs of a frog were perfused simultaneously.

Left leg With pituitrine	Right leg Without pituitrine
11 <sup>15</sup> Sciatic nerve cut.	11 <sup>16</sup> Sciatic nerve cut.
11 <sup>36</sup> Femoral artery opened.	11 <sup>42</sup> Femoral artery opened.
11 <sup>37</sup> Perfusion with gum Ringer + 1:50,000 pitui- trine begun.	11 <sup>43</sup> Perfusion with gum Ringer begun.
11 <sup>38</sup> Perfusion with gum Ringer + 1/3 ox cor- puscles + 1:5,000,000 acetyl-choline + 1:50- 000 pituitrine.	11 <sup>45</sup> Perfusion with gum Ringer + 1/3 ox corpus- cles + 1:5,000,000 ace- tyl-choline.

Acetyl-choline added to both perfusion fluids to maintain the arteries in a constant state of dilatation. It proves excellent for the purpose.

Perfusion pressure measured by a mercury manometer just behind each cannula. Mean pressure 10 mm. mercury in both systems.

11 <sup>45</sup> Slight dilatation. No sta- sis.	Somewhat more dilated. No stasis.
12 <sup>00</sup> Contracted again. About normal.	Dilated.
12 <sup>15</sup> Completely normal.	A little better, but distinctly dilated. Some narrow capil- laries, however.
1 <sup>05</sup> Perfusion pressure raised to about 20 mm. in both sys- tems, with no immediate effect in either.	
1 <sup>20</sup> Capillaries have con- tracted somewhat. Many of them are now very narrow. Melanophores spread out, color of leg nearly normal.	Capillaries greatly dilated.  Melanophores maximally con- tracted.
1 <sup>55</sup> No change. Perfusion fluid (25 cc.) used up.	Leg very pale. Numerous stases and bleed- ings.
2 <sup>05</sup>	Perfusion fluid (25 cc.) used up.

We have made a few perfusion experiments with suitable concentrations of adrenaline instead of pituitrine, but have failed to find any tonic influence of this substance upon the capillaries.

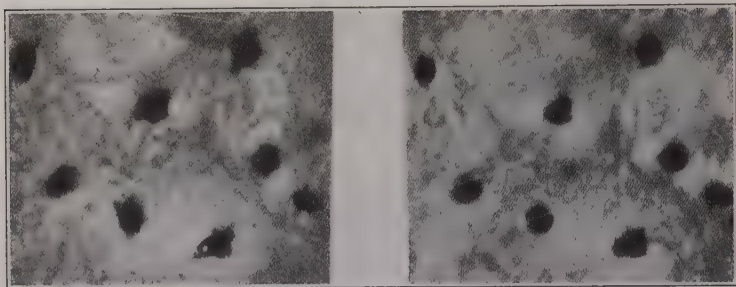
In perfusion experiments recently (1927) undertaken in my laboratory by Drinker the constrictor effect of pituitrine on capillaries could not be confirmed. Drinker tried both commercial pituitrine and extracts freshly made from dried posterior lobe (standard samples). The difference in technique, which may be responsible, is that Drinker's perfusion fluid was not made up with blood corpuscles. This point will, of course, require further experimentation, but our original results were so clear-cut and decisive that I have no hesitation in maintaining them as substantially correct.

A number of observations go to show that the pituitary hormone is normally present in the blood not only of frogs but also of mammals.

When in the brown frog the femoral artery is clamped, the current of blood in the web is usually diminished so as to be barely visible in the larger arteries, though it rarely stops completely. When the clamping is maintained for ten to twenty minutes both arteries and capillaries become gradually dilated and filled up with the blood flowing slowly in. The diameter of individual capillaries can be observed to increase from about 5 to about  $20\mu$ . At the same time the melanophores contract visibly (Krogh and Rehberg, 1924) as shown in Figs. 53 and 54 reproduced from our cinema film. When the clamp is removed a rapid current of blood passes through the dilated vessels, which, after five minutes, are again obviously contracted and may return to the normal state in ten minutes or less while the melanophores expand to their former dimensions. The reaction of the melanophores shows the pituitary hormone to be present in the circulating

blood and to be used up to a certain extent during the occlusion.

The reactions of the vessels may conceivably be due partly to metabolic products accumulated during the occlusion. In the corresponding reaction in mammals, which will come up for discussion presently, the "reactive hyperemia" is due to abnormal metabolites, produced when the supply of oxygen fails, but in the



Figs. 53 and 54. Black pigment cells in frog's web before and after stopping of the blood supply for 10 minutes.  $\times 270$ .  
After Krogh and Rehberg.

frog's web an excess of oxygen will diffuse in through the surface. The stagnant blood retains its bright arterial color and only turns blue when the atmospheric oxygen is shut off by means of a cover slip. If metabolites are, nevertheless, partly responsible for the vascular dilatations they must in the frog's skin be the normal metabolites produced in the presence of an excess of oxygen. However this may be, the experiment clearly indicates the presence of the pituitary hormone in the circulating blood.

In the experiments mentioned in a former chapter in which we had cut the femoral artery of one leg of a frog to get rid of the nerve plexus which might be present in its wall, we were able to follow the develop-

ment of the collateral circulation by the change in color of the leg. So long as the circulation was imperfect the leg was perceptibly paler in color than the other leg, which was normally provided with blood.

The presence of the hormone in the blood of mammals has been demonstrated by perfusion experiments on the hind legs of brown frogs (Krogh and Harrop, 1921). In these Harrop compared the action of gum Ringer and washed ox corpuscles with that of ox blood diluted usually to  $2/3$ . The difference was most marked. Perfused with Ringer the capillaries of the web began to dilate at once, perfused with blood they would maintain their tone for a period of an hour or even two hours. Ox blood and rabbits' blood were found to have about the same activity, while horse blood was more and pigs' blood less active in keeping up capillary tone. At the same time the blood would keep the melanophores more or less expanded, while they would contract to ball shape with Ringer. In the next series of experiments dialysate containing only the crystalloids of ox blood was tested and found to contain the active principle. Recently (1926) I have utilized the melanophore reaction in the hind legs of frogs to make determinations of the pituitrine content of horse serum in comparison with the international standard preparation of dried posterior lobe, of which  $1/2$  mg. has been accepted as the unit. The experiments showed the concentration in horse serum to be about  $10^{-4}$  international units per cc. while the concentration in blood from the jugular vein was about 50 per cent higher than in blood from the saphenous vein. The lowest concentration which would show a recognizable action on the melanophores was about  $10^{-6}$  unit per cc.

That pituitrine in such low concentrations has a very definite action also on mammalian and human skin capillaries has been amply demonstrated.



The first experiments were made by Miss Carrier (1922) in my laboratory. She found that introduction into the human skin of a minute drop (a fraction of 1 cmm.) of pituitrine (Parke, Davis) diluted even to 1/100 would cause contraction of neighboring capillaries without affecting the arterioles. The reaction

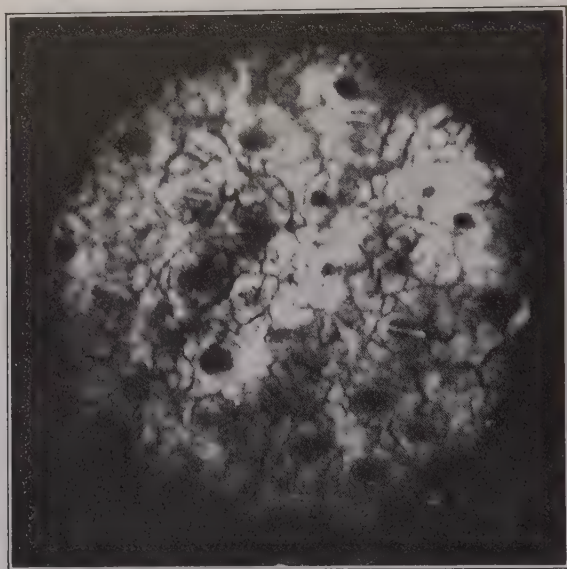


Fig. 55. Skin vessels in dog's ear before intravenous injection of 1 cc. pituitary liquid.  
After Kolls and Geiling.

would come on after a latent period of 2-3 minutes and last according to the concentration for 30 to 105 minutes. Heimberger (1925, 1) has confirmed these observations, but by using stronger solutions has obtained contractions of arterioles also. He describes how after pituitrine the capillaries under observation may show a series of rhythmical, more or less complete contrac-

tions with a period of about 5 seconds, while in the interval they are only moderately contracted.

The most important observations are those of Sacks (1924) who injected doses of from 0.03 to 0.005 cc. pituitrine (Burroughs, Wellcome) intravenously and obtained a definite blanching of the skin especially

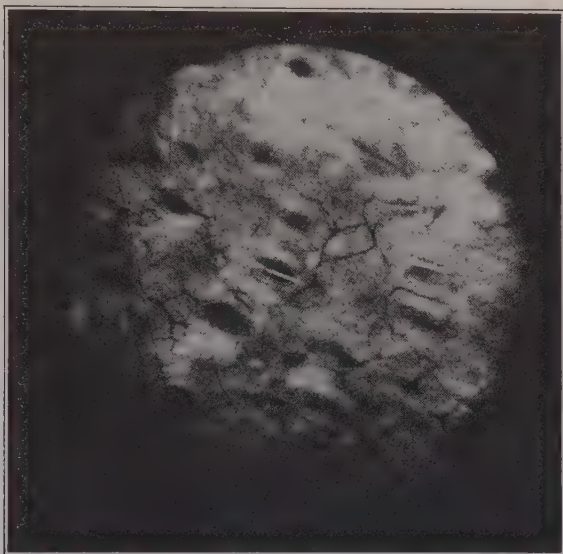


Fig. 56. Skin vessels in dog's ear  $2\frac{1}{2}$  minutes after intravenous injection of 1 cc. pituitary liquid.  
After Kolls and Geiling.

marked in the face, but also noticeable in the forearm, coming on after about 1 minute and lasting 20-30 minutes or more. 0.005 cc. is about one millionth of the blood volume, and the dose probably not even doubles the concentration of the hormone previously circulating. Sacks finds that the blood pressure is very slightly if at all raised by the doses employed and shows that

the arterial inflow to the forearm does not decrease during a visible pituitary pallor, thus demonstrating quantitatively that the arterioles, on which the inflow rate depends, remain unaffected.

Kolls and Geiling (1924) have observed a very pronounced constriction of skin capillaries in dogs of 8-12 kg. lasting, after doses of  $\frac{1}{4}$  to 1 cc. of Armour's pituitary liquid, from 15 to 91 minutes. The effect is well shown in the accompanying pictures (Figs. 55 and 56).

Florey and Carleton (1926) who ejected strong pituitrine from the fine opening of a pipette on to the mesentery of a cat submerged in a saline bath noted a complete closure of all the capillaries in the field while the arterioles were not greatly affected.

Poulsson (1926) has studied in my laboratory the effect of pituitrine injections on the blood pressure fall produced in cats by histamine and shown by Dale and his collaborators (Lecture III, p. 59) to be due to relaxation of capillaries. After moderate doses of histamine a sharp rise in pressure is brought about by the injection of a small dose of pituitrine. After larger doses the effect, though distinct, is less pronounced.

In a later paper (1926, 2) Poulsson has studied the pronounced inhibitory effect of pituitrine on experimental inflammation in the conjunctiva of rabbits, but these experiments will be more conveniently dealt with in a later lecture.

We have in adrenaline and pituitrine two hormones, normally present in circulating blood and capable of acting both on capillaries and on larger vessels, notably arterioles. What main function then do we have to ascribe to each? In my opinion this question is answered when we study the effect of minimal doses, corresponding, more or less, to the variations which may occur in the intact organism. A minimal dose of adrenaline, injected into the blood or added to a perfu-

sion fluid, normally diminishes the flow of blood to the organs studied, and such an effect is without doubt brought about by constriction of arterioles. Pituitrine, on the other hand, appears to have no effect in minute dose on the rate of inflow of blood, but a distinct effect on the blood color, brought about by constriction of skin capillaries and venules. So far as it goes the evidence, therefore, points to adrenaline as being the arteriolar and pituitrine as containing a capillary hormone, but it must be admitted that the action of pituitrine in physiological concentrations has been demonstrated so far only for cutaneous capillaries, and that those of other organs may react differently or not at all.

*Substances producing capillary dilatation.*

In experiments on the tongue of the frog I found that urethane shows a very powerful dilator action on capillaries, while it is indifferent toward arteries and arterioles. When a microscopically small drop (that is, a small fraction of 1 cubic millimeter) of 25 per cent urethane is applied to a capillary in the spread ventral surface of the frog's tongue a complete relaxation occurs. The capillary may become filled quite gradually from an arteriole which remains so narrow that the corpuscles are coming through one by one. The relaxed capillary may reach a diameter finally of  $50\mu$  and show a peculiar varicose appearance. In such a capillary complete stasis develops, and even after several days the vessel may remain filled with a mass of corpuscles. Application of 25 per cent urethane in larger quantity may produce a maximum dilatation also of the muscle capillaries below the mucous membrane, but this is accompanied by much twitching of the muscles, which may of itself cause dilatation of the capillaries. Application of 5 per cent urethane produces the same reaction in the superficial capillaries,

but it develops more slowly, and even a 1 per cent solution may produce a moderate dilatation when remaining on for several minutes. This latter concentration is only about twice to thrice as high as the urethane concentration in the blood of a completely narcotized frog (Krogh, 1914).

Experiments with urethane on the cocainized surface of the frog's tongue show that the effect of strong solutions, at least, is partly due to nerves. On the anesthetic surface the reaction develops much more slowly and, with 5 per cent urethane, the dilatation never becomes maximal and no stasis occurs.

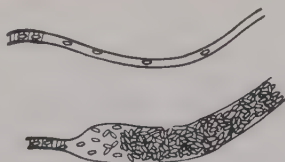


Fig. 57. Diagram of the effect of urethane on capillary in frog's tongue.

In the skin and web of the frog the action of urethane upon the diameter of capillaries is less striking, but a definite dilator action of a 5 per cent solution can be made out. In the bladder we have failed to observe any effect of urethane solutions up to a strength of 25 per cent.

The effects of the volatile narcotics, chloroform and others, on the tongue and web of the frog are similar to those of urethane, but chloroform shows in addition a very peculiar effect upon the red corpuscles, which become strongly adhesive to one another and to the capillary walls. Dale and Laidlaw (1919) found in their experiments on histamine shock that while unanesthetized cats will stand comparatively large doses



of histamine without developing shock, that is, without any extreme and irreparable dilatation of the capillary system taking place, anesthetized animals are vastly more susceptible. This point has been worked out by the British Committee on Surgical Shock (Medical Research Committee, 1919), who find both in experiments on animals and in observations on patients suffering from traumatic toxemia that the onset of shock symptoms is greatly accelerated and may be directly induced by anesthetization. They find, further, that the depth of the anesthesia is very important: the deeper the anesthesia the greater liability to shock. The kind of anesthetic used, chloroform, ether, urethane, seems to be irrelevant, but it is definitely stated that anesthetization with nitrous oxide gas can be used with impunity. These observations are to be understood in the light of the above experiments. The ordinary anesthetics have themselves a dilator effect upon capillaries which is just imperceptible by itself at the concentration inducing complete anesthesia, but is, nevertheless, sufficient to cause more or less complete loss of tone in the capillaries when its effect is added to that of some other capillary poison.

#### *Capillary poisons.*

As capillary poisons I put down in this section a number of substances acting in very weak solutions probably directly and more or less specifically on the capillary wall. Heubner (1925) has attempted a more detailed analysis of the action of a number of substances and distinguishes between substances acting solely on capillaries of which dionine is put down as the type, and others acting on capillaries and nerves (histamine), or capillaries and tissue cells (arsenic), or capillaries, nerves, and tissue cells (mustard oil).

While agreeing in principle to this classification,

which will form a most useful basis for work on pharmacological lines, I do not think it expedient to utilize it for my present physiological purposes.

As a type of substances which show a selective action on the contractile elements of the capillary wall Heubner studied (1907) the gold salt,  $\text{AuCl}_4\text{Na} + 2\text{H}_2\text{O}$ , and describes the effects of an intravenous injection of a lethal dose of this substance in mammals (rabbit, cat, and dog). The lethal dose for a rabbit is about 15 mg. per kg. body weight. For the carnivora it is about three times as high. The arterial pressure begins to fall during the injection and continues to fall, reaching zero in a few minutes (not above ten), when the animal dies, though the heartbeat may continue for a short time afterward. At autopsy the parenchymatous organs, as also the lungs and muscles, appeared to contain an abnormal quantity of blood, and bleedings from minute vessels were frequent. The intestines, especially the empty stomach, duodenum, and jejunum, of the carnivora were abnormally injected with numerous patches of a deep red in the mucosa, in spite of the fact that the animals had been without food for a day before being used for the experiments. In several cases considerable quantities of blood were found in the abdominal cavity, though no macroscopic wounds could be detected.

The microscopic examination of the organs showed dilated and very numerous capillaries and dilated venules and very numerous microscopic bleedings from capillaries and venules, especially in the liver, lungs, and kidneys. The small arteries were everywhere contracted, in most cases even to obliteration of the lumen.

These post-mortem observations, together with the sudden fall of arterial pressure preceding death, show clearly that we have to do with a relaxation of the capillaries and venules to such an extent that only a

fraction of the blood poured into them could return to the heart to keep up the circulation.

An even clearer picture of this reaction has been obtained by Heubner through microscopical observation of the mesentery of curarized frogs before, during, and after injection of gold salt into a femoral vein. By careful preparation Heubner secures a normal circulation in the mesentery. The injection of 0.5 cc. 0.1 per cent gold salt makes no immediate difference, but after one-half to one minute, when the poison reaches the mesentery, a very large number of new capillaries are quite suddenly opened up. The network of available capillaries is increased about three- to fourfold, and macroscopically the color of the exposed piece of intestine changes from pale to a distinct red. Very soon afterward the circulation becomes visibly slower and ceases altogether after a few minutes. The animal is "bled to death into its own capillaries," as Heubner himself aptly puts it.

A number of other substances act as capillary poisons. These include several double salts of heavy metals belonging to the gold and iron groups, arsenic, and the organic bases emetine and sepsine (Faust, 1904). Several of these show, of course, other toxic properties beyond that exercised upon the capillary wall, which latter becomes dominant only when they are introduced in suitable concentration directly into the blood. For some of them, especially when given in doses which are not immediately lethal, the capillaries of the intestine appear to constitute the place of predilection: they produce an enormous hyperemia of the mucous membrane with numerous capillary ecchymoses.

The most interesting of the substances having a pronounced dilator action on capillaries is no doubt histamine, particularly since, according to Lewis, this is

the substance normally responsible for the reactions classed as indirect and dealt with at length in the next lecture.

By means of histamine Dale and Richards (1918) made the beautiful study of capillary reactions referred to at length in my third lecture. It will be remembered, as the result of this study, that small doses of

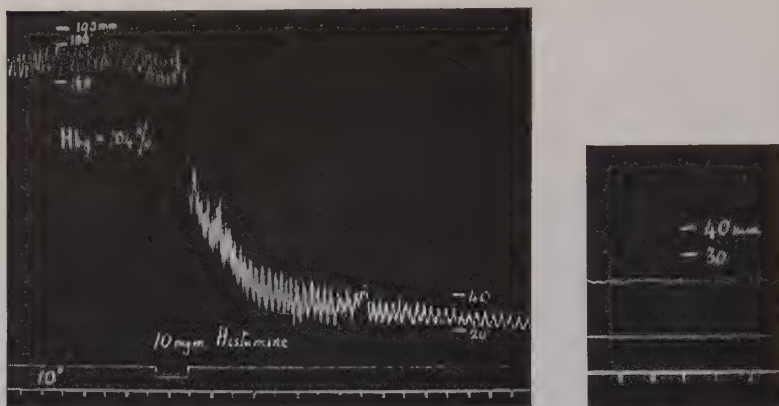


Fig. 58. Effect of 10 mg. histamine on blood pressure in dog.

Fig. 59. Continuation of Fig. 58. Eleven minutes after the injection.  
After Dale and Laidlaw.

histamine injected intravenously in cats (or other carnivora) produce an evanescent fall of blood pressure, which analysis showed to be due to capillary dilatation.

Dale and Laidlaw (1919) have investigated the even more striking effects of the introduction into the circulation of larger quantities of histamine. The blood-pressure changes resulting from the injection in cats of a single large dose (1 to 2 mg. histamine per kg.) are complicated by the effects of the poison on other organs, but the general effect is a very pronounced fall in arterial blood pressure, which reaches in a few

minutes a level of 50 to 30 mm. By a slow infusion of a dilute solution of histamine the initial irregularities of the action can be avoided, and the resulting blood-pressure curve shows an initial rapid fall in pressure, corresponding to that observed after a single small dose, and a subsequent slower decrease, during which the pulse waves become gradually smaller, until finally they disappear almost altogether. When the heart is inspected at this stage it may still be beating vigorously, but it drives very little blood into the systemic arteries, because the supply of blood through the systemic veins, which are found to be flaccid and empty, has failed.

At this stage an intravenous injection of a sufficiently large quantity of saline will bring about a rapid recovery of the output of the heart and a consequent increase in the blood pressure, showing that neither the force of the heart nor the peripheral arterial resistance has been impaired.

The blood, not being returned to the heart, must evidently be stored somewhere, and Dale and Laidlaw made a systematic search for it. As described above, it was absent from the great veins, and the arterial system was found to contain very little blood. "There remained the capillaries and venules, and here, at last, signs of accumulation of blood could be detected. The signs were clearest in the case of the abdominal viscera, possibly on account of the greater transparency of the tissues. If the abdomen was opened in the earlier stages of the main fall of the blood pressure, the intestines looked diffusely and somewhat duskily red, and the network of venules stood out distinctly. The pancreas had a purplish, congested appearance."

In the skeletal muscles no definite accumulation of blood could be made out in the ordinary experiments, but, when a state of plethora was brought about by



transfusion of blood from another cat, the general peripheral engorgement produced by histamine was very striking. The skeletal muscles were deep red and obviously contained much blood. There can be no doubt, therefore, that the muscle capillaries become relaxed by histamine, though they are probably able to withstand the toxic influence a little longer than the intestinal.

A very instructive comparison has been made between the effect of histamine and another "depressor" drug, acetyl-choline. By a carefully adjusted, very slow infusion of a dilute solution of acetyl-choline into the veins of a cat under ether it is possible to produce a persistent low arterial pressure of 40 to 50 mm. by vasodilatation alone without any complicating reactions. In such an experiment the output of the heart remained thoroughly efficient. The intestines showed a pink flush and pulsated obviously, and the great veins were well filled, but not overdilated. Perhaps the most striking contrast with the effect on the circulation produced by histamine, when similarly administered, was presented by the events following the stoppage of the infusion. After acetyl-choline the arterial pressure began immediately to rise from the low level, at which it had been maintained, and with an extraordinary rapidity regained or surpassed the level at which it had stood before the infusion began. After similar treatment with histamine the typical picture is the *circulatory "shock"* described.

Nothing can be more striking than this contrast between the result of arteriole dilatation and capillary dilatation, respectively.<sup>3</sup>

Studying the depilated ears of cats under the microscope by reflected light Hooker (1920) has been able to follow the capillary reactions after intravenous injection of a suitable dose of histamine. "The results were

uniformly clean-cut and decisive. Within a few minutes after the injection the capillaries and venules were filled with stagnant blood and definitely dilated. The dilatation was distinctly more conspicuous in the venules. These changes developed in conjunction with the fall in arterial blood pressure."

For the same purpose Rich (1921) has employed a very different method. Flooding the peritoneal cavity of cats with Zenker's fluid, he obtained a prompt fixation of the omentum in which the capillaries could afterward be studied microscopically. If the tissue was thus fixed immediately after the intravenous infusion of histamine, marked capillary and venous dilatation and engorgement could be demonstrated. This vascular change was entirely absent in control experiments in which salt solution was infused instead of histamine.

Miss Carrier (1922) in my laboratory has tested the local effect of histamine on the skin of man. At the base of the nail histamine (ergamine phosphate 1:1,000), introduced into the skin by means of a glass capillary a few hundredths of a mm. in outside diameter, produced some widening of the nearest capillaries with hastening of the stream. The increase in the current might be evidence for a dilatation of arterioles were it not that the arterial loop of the nail capillaries is often so narrow that a definite increase in its diameter may very well diminish the resistance, especially when other capillaries, supplied from the same arteriole, are not simultaneously dilated. When applied in the same way on the back of the hand, the introduction of this minimal quantity of histamine gave rise, after a latent period of a few seconds, to a sharp, painful itching, lasting one to five minutes and accompanied by a definite reflex erythema over an irregular area of 3 to 12 sq. cm.

The local reactions of the human skin to histamine

have been most elaborately studied by Thomas Lewis, who emphasizes the "triple response." When histamine (1-3,000) is punctured into the skin by pricking through a drop with a fine needle, a small red spot develops after about 20 seconds around the puncture, a few seconds later the red flare due to the axon reflex mentioned in Lecture VI begins to develop, and after 1-2 minutes a small wheal appears, exactly covering the red spot. This grows during a few minutes and is at first pink, paling after a few more minutes and subsiding after 30-60 minutes. Stronger solutions act perhaps a little more quickly. The lowest concentration which can be distinguished from saline is 1-500,000.

The local red spot is due, as mentioned above, to a dilatation of the minute vessels and especially of the venules. This local dilatation is equally well developed on denervated skin, and after occlusion of the circulation relaxation will take place just as when the circulation is normal, and the relaxed vessels will be filled and dilated from the surrounding veins in spite of the very low pressure. The wheal is produced by exudation from the minute vessels which become permeable to plasma. Lewis has shown that the formation of a wheal in a few minutes requires a considerable local increase in blood flow brought about by arteriolar dilatation. He has shown further that the wheal fluid contains nearly the same amount of protein as the corresponding plasma. We shall reserve the discussion of this permeability for a later lecture.

The triple response of the human skin is the common reaction to a large number of very different stimuli having that in common that they damage tissue cells. As we shall see in the next lecture Lewis has been able to show that in all these cases the reaction of the vessels is secondary and due to the liberation of a specific stimulating substance from the tissue, while in the case

of histamine here under discussion it is assumed by Lewis that we have to do with a primary or direct reaction on the part of the vessels. This conclusion seems to be mainly based on two properties by the combination of which histamine appears to differ from the large number of substances showing a dilator action on the smallest blood vessels. The first and most important of these properties is its activity in extremely minute concentration and dose of which more examples will be given in the next lecture. This property is no doubt shared by certain poisons of animal and vegetable origin (e.g., those produced by mosquitoes and nettles), but these act more or less specifically on certain animals and tissues, while it is contended that the action of histamine is not confined to single species, but is common to all mammals and perhaps to all warm-blooded animals. Ebbecke (1923, 2) emphasizes the fact that histamine reactions are not reduced by repeated application, while the specific poisons (e.g., of nettles) will induce a more or less complete immunity.

Until recently it was generally assumed that certain mammals, including rabbits and guinea pigs, were not susceptible to the action of histamine. In the case of rabbits it has been found by Feldberg (1927) that histamine introduced into the general circulation has a dilator action on normal capillaries and venules just as in other mammals, while the effects on the general circulation are complicated by a strong constrictor action on arteries. Lewis and Marvin (1927, Lewis, p. 109) have produced the dilator effect on the minute vessels of the rabbit's ear by pricking in histamine in dilute solution. In this case, however, the "reflex flare" is absent, as would be expected, and, probably owing to the arterial constriction, no wheal develops.

Although statements have been made to the contrary

(Doi, 1920; Killian, 1925; and others) I feel confident that histamine even in high concentrations has no action on frog's capillaries. We have tested it locally on the web, tongue, and muscles, both on the intact surface and after introducing it below the epithelium in various concentrations up to that obtained by placing a minute crystal of ergamine phosphate on the surface and surrounding it by pricks with a fine needle. Rehberg and I, and later Drinker, have made perfusion tests by adding histamine to perfusion fluids known to maintain capillary tone, but have failed to find any action.

Even in those mammals where histamine is undoubtedly active, its dilator action on the capillaries depends upon certain conditions which are, I believe, far from being completely understood.

It will be remembered, from the third lecture, that Dale and Richards found that the dilator action of histamine could be demonstrated in perfusion experiments only when the perfusion fluid contained enough red corpuscles to provide an adequate supply of oxygen and, in addition, a small amount of adrenaline, but failed to appear when these conditions were not fulfilled. Dale and Richards ascribed this failure to loss of tone of the capillaries, but the explanation appears to me very doubtful, since they were able by means of their beautiful technical arrangement to change without interruption from the normal circulation of the animal to the artificial perfusion, and the "loss of tone" or spontaneous dilatation must take some time to develop. Burn (1922) has shown that after section and complete degeneration of the nerve fibers to one leg of a cat the limb does not show the usual plethysmographic response—increase in volume—to a small dose of histamine intravenously administered, but shows passive contraction, due to the fall in arterial



blood pressure, instead. By depriving a leg of the sympathetic innervation alone Burn was able to show that lack of sympathetic tonus did not interfere with the normal response, and he concludes, therefore, that capillary tone has been seriously impaired by the interruption of the connection with posterior root fibers. Though the circulation in the leg deprived of its posterior root fibers may very well have suffered, any considerable dilatation of capillaries cannot, I think, have taken place, because that would involve a change in appearance which could not have escaped notice, the more so as the pads of the foot were regularly inspected; they would, at the very least, have become of a deeper red color. There appears, therefore, to be something beyond loss of tone which is able to prevent the response of capillaries to histamine.

Histamine is normally produced in the mucous membrane of the small intestine (Barger and Dale, 1911). It is a common impurity in tissue extracts (Abel and Geiling, 1924), and recently Best, Dale, Dudley, and Thorpe (1927) have succeeded in isolating the base in comparatively large quantities from the liver (0.005 per cent) and especially from the lung (0.07 per cent), and Lewis (p. 235) has demonstrated in the human skin a natural vasodilator substance, acting like histamine, the concentration of which, calculated as histamine, would be about 0.016 per cent. These facts, of course, speak strongly in favor of the view that histamine may be *the* dilator substance for capillaries regularly acting in the organism of warm-blooded animals, while we have to assume that a different substance is produced and is active in the same way in frogs.

It would be natural to call these substances dilator hormones, and Burn and Dale (1926) accept histamine as a regular hormone liberated mainly from the lungs,

but the evidence for this is inconclusive, and it appears more likely that they are liberated from various tissue cells and act locally on the capillaries in the immediate neighborhood. This activity will be dealt with in some detail in the next lecture.

### NOTES

<sup>1</sup> In the first edition of this book (1922) a rather elaborate description was given of methods used and experiments made in my laboratory to establish the existence and study the action of the capillary hormone. Owing to pressure of other work, upon which the whole of the laboratory staff became engaged, these studies were never published in the normal way—as papers in scientific journals—and we prefer now to consider them as published in the first edition referred to. In the present edition I propose to give only a summary of the evidence and to refer readers who may be interested in details to that book, which will be sufficiently accessible in laboratories and libraries.

<sup>2</sup> A very complete account of the morphology and physiology of the pituitary gland is to be found in the book of Houssay (1918). The influence of the pituitary on the melanophores of frogs was discovered by Hogben and Winton (1922) independently of Rehberg and at the same time.

<sup>3</sup> For many years the circulation was regarded as if the heart action and the vasomotor (arterio-motor) mechanism were the only variable factors, and as if any failure or depression not due to the former must be due to the latter. Observations by Yandell Henderson (1908) upon an experimental form of shock first demonstrated clearly the occurrence of what he termed failure of the “veno-pressor mechanism,” characterized by decreased and finally inadequate venous return to the right heart, resulting in decrease in cardiac filling and discharge, while the arteries at the same time were found to be, not relaxed, but constricted. Other papers by Henderson and his collaborators (1909, 1910) brought forward evidence in favor of the view that it is this mode of circulatory failure rather than “vasomotor failure” which is characteristic of shock and, although there are a number of matters still needing further elucidation, this conception has now come to be generally accepted.

## LECTURE X

### INDIRECT CAPILLARY REACTIONS

THE idea that stimuli might act primarily on tissue cells, the altered activity of which influence in turn the blood vessels, was first put forward by Ebbecke (1917) and further elaborated by him in 1923 (1 and 2), on a number of examples which place it beyond doubt that such a mechanism exists, but the close study that has elucidated the essential features of this mechanism has been made by Lewis and his collaborators.

#### *Acute Reactions.*

Lewis shows that in the human skin a large number of stimuli, which have this in common that beyond a certain strength they definitely injure the tissue, call forth the triple response just as it is produced by the pricking in of an infinitesimal dose of histamine. These injurious stimuli include burning heat, which is experienced according to Lewis when the skin temperature is raised above  $42^{\circ}$  to  $43^{\circ}$ , freezing of the skin, mechanical injuries, and the stimuli produced by a number of very diverse chemical substances such as acids, alkalis, metal salts, formaldehyde, morphine, nettle poison, peptone,<sup>1</sup> and special foreign proteins in susceptible subjects. The sequence of events after all these stimuli is the same: considerable local dilatation of the minute vessels after a short latency, surrounding flare through axon reflex, local whealing due to increased permeability of capillaries and venules, and increased blood

flow through them. When the stimuli are properly graded so as to produce for instance the same degree of whealing the time relations of the responses will also be remarkably similar as shown by the following example:

Zero time	Stroke	Histamine punctures
After 20 secs.	red line begins	red spot seen
After 30 secs.	flare begins	flare begins
After 70 secs.	wheal begins	
After 80 secs.		wheal begins
After 3 mins.	wheal almost full and pink	wheal almost full and pink
After 8 mins.	wheal pale	wheal pale
After 47 mins.	wheal pale and diminishing	wheal pale and diminishing

The conclusion is that all these stimuli act through a common mechanism, a substance liberated by the injured tissue cells, and this conclusion is strengthened and elaborated by a series of further observations and experiments.

A certain number of persons are specially susceptible to one special form of stimulation. There are cases in which the skin is abnormally sensitive to cold or to heat (Duke, 1924). There are a fairly large number of cases sensitive to special proteins or protein derivatives, and there are certain persons showing "urticaria factitia" in which a comparatively slight mechanical stimulus, such as a single stroke with a blunt point, will evoke the full triple response. Lewis shows that in all such cases the vessels exhibit no special reactivity, but respond normally to histamine and other stimuli. The abnormality must be in the tissue cells, and the natural inference is that these liberate on special provocation an abnormal amount of the substance postulated. As will appear from the following Lewis

has made extended use of cases of urticaria factitia for the study of these indirect reactions.

When in a susceptible person a stroke that is just adequate to produce a red line is laid down on an arm after occlusion of the circulation the red line will persist during the whole period of occlusion (tested up to 25 minutes), while with normal circulation it fades, sometimes completely, during the same period. This is interpreted as due to the substance being retained during occlusion and washed away by circulation, but it is evident that the observation is open to other interpretations as well. It is far more important that during the occlusion a spreading of the reaction takes place which can only be due to diffusion or a slow flow of a substance. Lewis has compared *during occlusion* the gradual increase in the area affected after a histamine puncture and the corresponding broadening of the line affected after a stroke in a susceptible person and found them very similar.

By occlusion the appearance and, what is more important, the fading of the surrounding flare are postponed practically for a period corresponding to the duration of occlusion, no matter whether the reaction is brought about indirectly or directly by the injection of histamine, as illustrated by the accompanying Fig. 60. The following experiment furnishes specially clear and conclusive evidence: Two similar stimuli (histamine punctures, strokes, freezing, etc.), are simultaneously applied to distinct points, one above the other, in the same arm, and equal flares and wheals are allowed to develop. When this has happened, an Es-march bandage is tightly wound several times around the arm, so that it includes the lower wheal. The bandage is maintained in position and the circulation to this wheal thereby stopped for as long as 20 minutes. That part of the corresponding flare which is visible



above the bandage retains its original extent and color throughout the experiment, while the upper control flare fades away. This shows that in all the cases some-

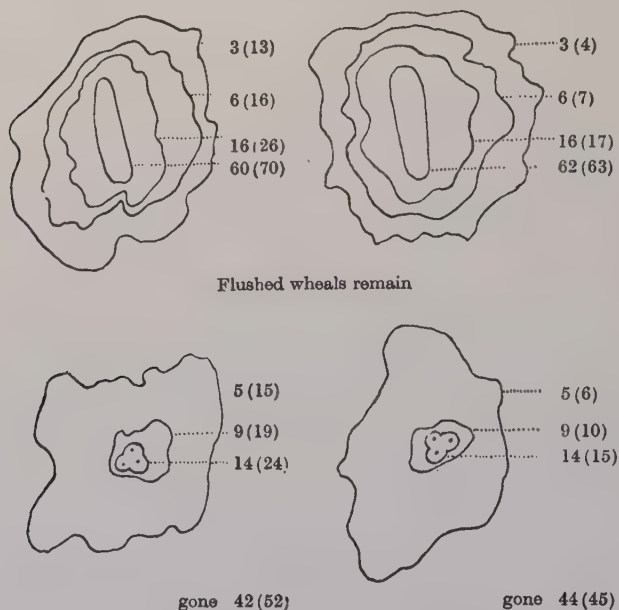


Fig. 60. Urticarial subject. The vessels of the two arms were occluded. One minute later the left forearm was stroked (above), and a group of three histamine punctures (1 in 3,000) was laid down (below); at the tenth minute, or 9 minutes later, the right forearm was similarly and symmetrically treated, both arms being released at the eleventh minute. The contours of the areas of flare are shown as these fade away. In the stroke figures, the fading was followed until the redness became confined to the wheals. After Lewis and Grant.

thing must continue to act on the nerve endings below the bandage throughout the occlusion, and it must be concluded that it cannot be the original stimulus which is responsible, but a substance capable of being washed away by the circulating blood.

The evidence brought together by Lewis and here briefly outlined proves, in my opinion definitely, that we have to do in all the acute indirect reactions with a substance released by the injury to tissue cells, and makes it very probable that this substance is closely related to histamine, and may be histamine itself,—a conclusion already expressed by Ebbecke (1923, 1, p. 210).

On this latter point Lewis submits further and very instructive evidence. Studying the effect of small subcutaneous doses of histamine (0.3 mg.) on the general circulation of man he obtains a general flush of the skin, a rise in skin temperature of 1-2° C., a small decline of systolic, a greater decline of diastolic pressure and an appreciable rise of pulse rate. Smaller doses (0.06 mg.) still yield a perceptible flushing of the face and rise in general skin temperature (0.5°), but are unable to produce a distinct fall in blood pressure or any appreciable rise in pulse rate. Cases of urticaria factitia react in the same way to histamine injections, but in these persons quite similar general reactions could be brought about by stroking and whealing considerable areas of the skin of the trunk. Computing the amount of fluid transuded into the wheals and taking the substance liberated to be histamine the concentration was calculated as 1 in 1,500,000. When fluid directly collected from a stroke wheal was introduced into the skin small wheals could sometimes be produced though more often the result was negative. As mentioned above histamine cannot be relied upon to produce a visible reaction in concentrations below 1 in 500,000.

*The reaction to increased temperature.*

When an arm is immersed in a water bath at 41° C., the skin temperature will rise and after a few minutes

will become stationary at about  $39^{\circ}$ . The immersed skin will become red and, provided the arm is kept still, there will be a sharp line of demarcation between the heated and unheated skin. Lewis shows that this reddening which requires a minimum temperature of  $36^{\circ}$  to  $38^{\circ}$  is, mainly at least, due to the release of a vasodilator substance in the skin. The evidence is obtained by the two following types of experiment.

"The two arms are immersed in water at  $41^{\circ}$  to  $42^{\circ}$  and are there held still for 2 to 3 minutes: they become reddened to the water lines. The circulation to one is arrested and both are at once withdrawn and plunged more deeply into water at  $20^{\circ}$ . After an interval of usually 4 to 6 minutes the heat hyperemia has disappeared from the arm in which the circulation is intact. The other arm is now released and, when the reactive hyperemia consequent upon the release of the obstructed circulation has faded away, the heat hyperemia on this arm usually becomes visible and remains so for a period of about 6-8 or more minutes from the release."

In experiments of the second type, "the two arms are immersed in water at  $41^{\circ}$  to  $42^{\circ}$  for 2 minutes, are taken out and reimmersed more deeply, the one in water at  $30^{\circ}$  to  $34^{\circ}$ , the other at  $20^{\circ}$  to  $25^{\circ}$ . The fading of the lines of heat hyperemia on the two arms is then watched and timed. Now, if the heat hyperemia is due to a direct effect of warmth on the vessel walls, then this effect should be counteracted more quickly by the colder bath. The reverse is actually and very definitely the case, the heat redness disappears in about 2 to 3 minutes from the arm in the warmer bath, in about 5 to 8 minutes from the arm in the colder bath"; because the vasodilator substance is washed away more quickly by the greater circulation at the higher temperature.

Lewis concludes from the fact that redness is produced at temperatures which are only slightly above

normal and in no way injurious that the vasodilator substance must be a normal metabolite, and since there is every gradation between this response and the full triple response, produced by the application of burning heat, he concludes further that the response is in every instance due to the release of his H-substance, which must, therefore, be a normal metabolite. This point will come up for discussion in a following lecture, and I hope to show that lack of oxygen is the factor responsible for the liberation of a vasodilator substance at moderately increased temperatures.

### *Slow reactions.*

Certain stimuli call forth definite vascular reactions in man and animals after a period of latency of many minutes and even hours. A very instructive example has been given by Ebbecke (1923, 1), who introduced an isotonic sugar solution by iontophoresis into the human skin. Several minutes later redness develops and is accompanied by itching and wheals are slowly raised and coalesce. When the wheal subsides after a couple of hours the redness persists and even after several days the minute vessels are hypersensitive to stimulation. The mechanism of such slow reactions has been extremely difficult to understand, and the demonstration by Ebbecke and Lewis that we have to do in these cases with a primary injury to tissue cells and the secondary release of a vasodilator substance (or vasodilator substances) is of the greatest importance.

The influence of *light* upon the human skin and also upon certain lower organisms was carefully studied by Niels Finsen (1900) who showed that the ultraviolet rays of the sun were powerfully active, while some activity could be detected in violet and blue rays from the visible spectrum. The reaction of the skin is an erythema, a dilatation of capillaries and venules, with

increased temperature. In an experiment in which Finsen exposed his own arm, of which certain areas were protected by colored and clear glass plates, for 20 minutes to the light of a 40,000 candle power arc lamp, the erythema began to appear in the uncovered parts after 3 hours, reaching a maximum in 12 hours, decreasing again after 2 days, disappearing gradually in about 10 days, during which desquamation of the epidermis took place and was followed by the well-known brown pigmentation. This pigmentation faded very slowly and even after five months the areas corresponding to the glass plates could still be discerned. After about six months the arm was uniformly white, but even then a definite after-effect of the light upon the skin capillaries could be made out: when the arm was rubbed the skin became red, but the redness was less pronounced in the areas which had been covered during the experiment. The light had produced an increase in excitability of the capillary wall toward the mechanical stimulus of rubbing.

Two of Finsen's pupils, Dreyer and Jansen (1905), studied the light-erythema on the frog's tongue, excluding the temperature effect by filtering the light and irrigating the tongue with cold saline. They screened off the light by tinfoil, exposing only an area of 2.5 x 5 mm. for periods varying from about 5 to 30 minutes. In an exposed area all vessels became dilated in a few minutes and in the capillaries stasis developed rapidly. The limit between the exposed area and the unexposed surrounding was very sharp, any vessel crossing the frontier showing a sudden decrease in diameter to the normal width.

In a similar experiment Jansen (1906) pressed the frog's tongue between quartz plates, so that it remained quite bloodless during the exposure. The normal reaction—dilatation and stasis—developed in the



exposed area when the blood was again admitted afterward.

In the transparent tissues of frogs a normal circulation can only be maintained under strong illumination when the violet light of the visible spectrum is cut out by a suitable filter. The tissues must, therefore, be extremely sensitive to light and react seemingly without any latent period.

Lewis, working with the powerful ultraviolet radiation of the mercury vapor lamp, finds a much shorter period of latency, 30-60 minutes with short exposures of 3-6 minutes and even less with longer exposure. The reaction which is at first strictly limited to the exposed area of skin consists in a dilatation of all the superficial vessels with some increase in blood flow. The reaction occurs equally in denervated skin though it may be modified to some extent by nervous influences, as shown, for instance, in experiments by Dreyer and Jansen who cut through the cervical sympathetic on one side on albino rabbits and then exposed two small spots on each ear to concentrated chemically active light, one pair for ten, the other for thirty, minutes. The spots were made anemic during the exposure and carefully cooled. As in man the visible reaction began after several hours' latency, but always first on the side where the sympathetic had been cut. With the ten minutes' exposure the reaction on the operated side became very pronounced, while it was very slight on the control side. With the long exposure the reaction, though beginning much later on the control side, became quite as strong after about one to two weeks, and the authors note that the final restitution was always reached about one week earlier on the operated side. The results of the nerve section experiments are probably to be explained by the vasodilatation resulting from the cutting off of tonic impulses. The pressure in the arterioles and

capillaries will be abnormally high, and they will give way earlier when weakened by the light.

Lewis finds that the normal triple response can be evoked by irradiation indicating that his H-substance becomes liberated from tissue cells. It must be admitted, however, that with short exposures the wheal-

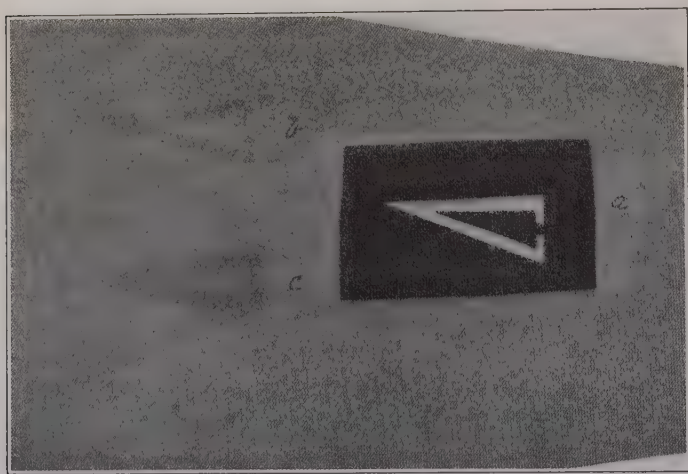


Fig. 61. Photograph of a forearm. *a* A black paper shield used in the following exposures. *b* Area of skin irradiated for 6 minutes, 4 hours before photographing. *c* Area of skin irradiated 6 minutes, 28 hours before photographing. After Lewis.

ing is usually very slight, while long exposures are prone to induce blistering, and of the surrounding flare there is generally only the merest trace, and even this may fail to appear. Lewis concludes that these discrepancies are due to the slow development of the reaction, the whealing being balanced by absorption through lymph channels and the flare by contractions of the minute vessels in response to the increased blood supply.

The main argument for the liberation of an active substance is the "diffusion flush" observed by Lewis and Zotterman (1926). Exposing the arm through the shield (Fig. 61a) they find that the reaction will change during 24 hours from the form b to the form c, showing a slight extension in all directions. With longer exposure the diffusion flush becomes even more pronounced and will often show extensions up the arm which correspond apparently to lymph channels (Fig. 62). When the reaction subsides the diffusion flush vanishes; it does not produce whealing, and only the



Fig. 62. Extension of flush 26 hours after ultraviolet radiation of area marked *u. v.* After Lewis.

area directly exposed will later become pigmented. Lewis has found that a diffusion flush similar in every respect will often develop after freezing and after stroking in susceptible persons. It usually comes several (10-40) hours after the original lesion.

Lewis is of opinion that we have to do in all cases with one and the same H-substance and cites in support the cases observed (e.g., Duke, 1924) in which the full triple response is called forth by sunlight in a few minutes. In the normal light reaction the rate of production of this substance is supposed to be very slow at first (ac in the diagram Fig. 63) and then to reach

a level, while in the acute lesions there is an initial large liberation (ab) falling off to essentially the same level.<sup>2</sup>

I think that the evidence points rather definitely to the existence of at least two substances, one of which, that may be histamine itself, possesses a relatively small molecule and is readily taken up by the blood through the capillary walls as shown by the general symptoms produced by the stroking in the experiment quoted on page 213. The other substance, predominant

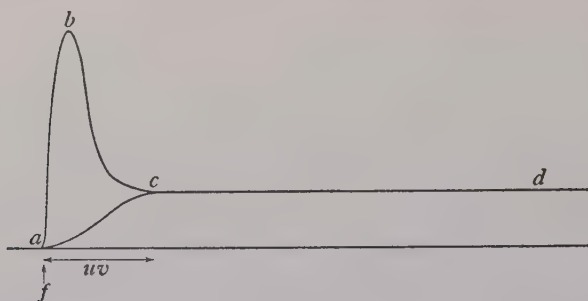


Fig. 63. Diagram of H-substance production according to Lewis.

in the reaction to ultraviolet light, must be one of extremely low diffusibility, practically unable to pass through the capillary wall. If histamine were produced at a slow and regular rate in an area after exposure to light its concentration must remain extremely low in the tissue spaces, because it must diffuse in through the enormous surface of the dilated blood vessels and be carried away. The formation of a diffusion flush surrounding the exposed area involves the presence of the active substance in fairly high concentration, such as can be attained only if the substance is taken up by the blood with extreme slowness or not at all.

I picture the process as a disintegration of cells in which histamine is certainly set free, and along with

that much more complex substances of a colloidal nature which have a similar action on the minute vessels, with the possible (nay, probable) exception that they do not stimulate sensory nerve endings and, therefore, fail to produce a flare. Lewis, who definitely admits the possibility that there may be more than one H-substance, mentions the fact that after heavy stroking the initial triple response may subside and the red line may even fade away, to a greater or less extent to return with renewed intensity later, when it may last for hours, while a flare fails to reappear.<sup>8</sup>

When the skin of man is exposed to strong light at any time during a certain period after a light-inflammation, the reaction is greatly weakened. This is usually ascribed to the resulting pigmentation, which keeps out the light from the sensitive tissue elements. This is not the whole truth, however. C. With (1920) has made some interesting experiments on the reaction to repeated treatment with the Finsen light of vitiligo areas in which no pigmentation develops. Even here a definite immunity to the light followed the inflammatory reaction due to the first treatment.

The reactions to X-rays and radium are according to Lewis of the same general type as those to light and a liberation of H-substances must be assumed in these cases also. The period of latency is, however, still longer, being usually from 2 to 5 or more days in the case of X-rays, and from 2 to 3 or more weeks in the case of radium. Lazarew and Lazarewa (1926) have observed that after exposure to X-rays the skin vessels will show for a very long time increased sensitivity to dilator stimuli and decreased to constrictor.

Certain chemical substances give rise to slow reactions. They are probably taken up and held by tissue cells and cause a slow degeneration and disintegration with liberation of H-substances. Lewis discusses the



effects of mustard gas (dichloroethyl sulphide) which after several hours causes deep reddening of the skin, whealing followed by blistering, and in certain cases ulceration and loss of tissue. Just as in the ultraviolet burn a flare often fails to be manifested or is indistinct, and after the reaction the skin becomes strongly pigmented for a period of many months.

A very typical case of a slow reaction is that following the application of abrine to the rabbit's conjunctiva studied by Ricker and Regendanz (1921). 0.01 mg. in 0.1 cc. saline is instilled into the conjunctiva. The eye is kept open for five minutes, during which time the fluid disappears. There is some hyperemia and slight edema of the surface. All the vessels which are microscopically visible are dilated. The current is rapid. This initial effect is comparatively slight. There is no stasis, but the substance evidently penetrates into the tissue and affects also the deeper vessels.

Eight hours later there is macroscopically a pronounced hyperemia and slight edema of the conjunctiva. Microscopically all the visible vessels are strongly dilated. In some parts of the surface there is capillary stasis, in all others a slow current. The next day the conjunctiva contains pus and is very hyperemic. Stasis has developed in almost all superficial capillaries and a number of capillary bleedings have appeared.

During the next two days the symptoms of inflammation, especially the bleedings, increase, but thereupon recovery processes set in, and after fifteen days the appearance of the conjunctiva is practically normal. A second instillation of 0.01 mg. abrine undertaken on the fifteenth day gives rise to even more pronounced inflammatory symptoms, which even thirty-two days later have not returned completely to normal,

in that the capillaries, especially in the deeper layers, are still dilated. The application of adrenaline at this stage produces an immediate hyperemia, but after two or three minutes the constrictor effect on the arteries of the deeper layer asserts itself.

That the tissue cells themselves are strongly affected by the poison is evident from the fact that the cornea becomes opaque and new capillaries develop and grow into the periphery of the cornea.

Lewis points out that bacterial poisons introduced into the skin—in many instances at least—produce slow reactions in every way comparable to ultraviolet light burns and describes the reaction to the intracutaneous injection of a small quantity of streptococcic or diphtheric toxin. The response develops within a day with streptococcic or 2 to 3 days with diphtheric toxin in susceptible persons. "The color of the skin becomes of an intense pink, not scarlet, and it becomes duller as it fades; it is accompanied by, and is coextensive with, a swelling of the skin. Signs of a surrounding arteriolar flare are indistinct, or, more usually, absent. The local reddening stands out brightly on skin rendered blue by a congestion test. The temperature is raised locally, though rarely by more than  $0.5^{\circ}$  C., and there may be tenderness. If the circulation to the limb is stopped and the blood is massaged out of the vessels, it returns immediately, and the original contour of highly colored skin is at once resumed. It is due, therefore, to an active dilatation of the minute vessels."

These observations have a very important bearing on the phenomena of inflammation to be discussed in some detail in a later lecture.

### *Reactive hyperemia.*

Reactive hyperemia is the term generally used to denote the dilatation shown by blood vessels when the tis-

sues have been deprived of blood for some time. The phenomenon is well known to surgeons who use the tourniquet or Esmarch bandage and was described as early as 1872 by Cohnheim. It was studied by Bier in 1897, and recently Lewis has made a number of observations and experiments on man from which he concludes that his H-substance is responsible for the reaction in its entirety. Being unable to accept this conclusion I shall begin by describing without comment a series of facts chiefly obtained by Lewis.

When the circulation to one arm is suddenly occluded and the occlusion maintained for a certain time of not less than one minute the arm will show a pink flush on release of the circulation and this flush will fade away gradually, lasting, as Lewis has found, half to three-quarters as long as the arrest in an arm maintained at 33° C. With longer arrest the period of flush is usually relatively shorter. Arrests lasting 20 minutes or more are associated with a good deal of discomfort, with numbness and aching during and with intense tingling and sometimes painful cramp soon after the release. A small area of skin can be deprived of circulation by pressure for long periods without danger or discomfort, and in a case given by Lewis such pressure exerted for 100 minutes was followed by about 40 minutes' hyperemia.

Lewis has studied also by plethysmographic methods the arm volume during and after circulatory arrest. With certain limitations and especially in short periods the volume is an accurate index of the state of dilatation of the vessels generally and of the minute vessels, which contribute most to the total blood volume, in particular. The curves (Fig. 64) show that even after very short arrests down to five seconds there is a definite period of increased volume, a hyperemia, which is, however, too slight to become manifest as a flush and

is perhaps confined to the muscles. After longer arrests the volume of the occluded arm remains increased for nearly the same period as the flush can be observed.

By means of his plethysmograph, supplemented by a cuff congesting the veins, Lewis has measured finally the rate of arterial inflow into the arm in the normal state and after occlusion. The initial rate is greatly increased and the more the longer the occlusion has

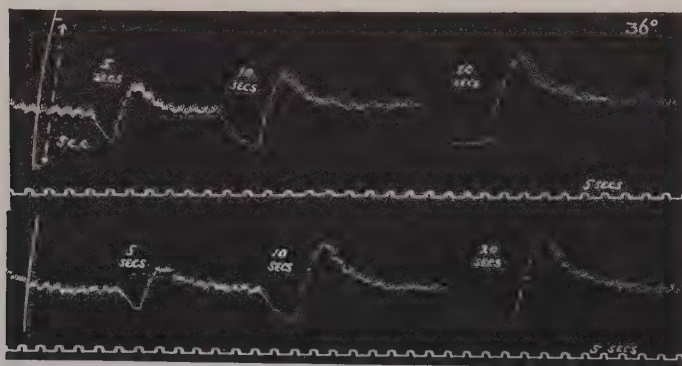


Fig. 64. A series of forearm volume curves. The subclavian artery was closed for the number of seconds shown and released.  
Arm volume 610 cc. After Lewis.

lasted as shown in Fig. 65. After one-half minute's occlusion the rate has risen from the normal 4 cc. per minute to 15 cc. and after 15 minutes' occlusion even to 55 cc. or 14 times the normal. This rate declines rapidly during the hyperemia as shown in Fig. 66, where it is at first 50 cc. per minute but reaches the normal value of 2.4 cc. after 5 minutes.

The experiments described show that both the minute vessels, capillaries, and venules, and the arterioles are involved in the reactive hyperemia. Lewis has observed that the visible flush is very strictly limited to



the area occluded and does not spread one millimeter beyond it, and he concludes that no arteries larger than the terminal arterioles can be involved. As shown on p. 141 this conclusion is invalid. The whole system of larger arteries can be dilated and the blood flow greatly increased without any visible effect being produced in the skin. It is practically inconceivable that increases in blood flow of the magnitude (beyond 20

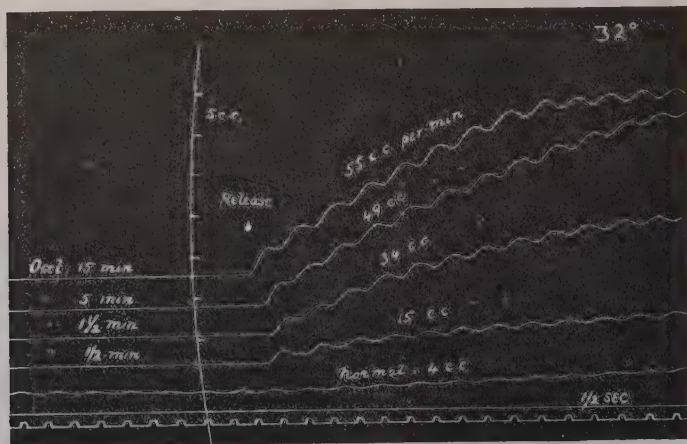


Fig. 65. Rates of inflow into occluded arm in normal conditions and after variable periods of circulatory arrest. Figures give rates per 100 cc. of tissue. After Lewis.

times) observed by Lewis can be brought about without dilatation of larger arterioles, and further reasons will be given below for the view that the whole arterial system is involved. Lewis, himself, has shown that the large subcutaneous veins of the arm or hand also show a considerably diminished tone in reactive hyperemia as indicated by their increased diameter under a given pressure.

A reactive hyperemia is induced not only when the



blood flow to a limb is occluded entirely, but also when it is merely reduced as in venous congestion. The brightness and duration of the flush will depend both upon the pressure thrown upon the veins and the time during which this pressure is maintained, and similar

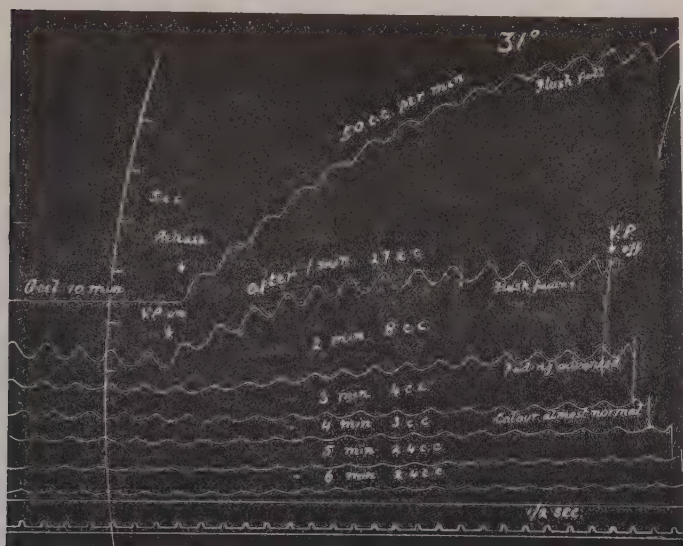


Fig. 66. Rates of inflow into occluded arm after circulatory arrest. The top curve was obtained as in Fig. 65 after 10 minutes arrest of the circulation. The remaining curves were each taken by releasing the original collecting pressure of 60 mm. Hg. (at *v.p. off*) and re-imposing it (*v.p. on*) at successive intervals of one minute. Rates per 100 cc. of tissue. After Lewis.

relations are found for the arterial inflow to the limb measured immediately after the reduction of pressure. Special experiments made by Lewis show conclusively that the dilatation of the vessels is active and is not simply due to the increase in pressure during the congestion.

Experiments by Rehberg and myself (Krogh, 1922) go to show that a hyperemia of the same type can be induced in the rabbit's ear by oxygen lack. The animal breathed from a small closed circuit respiration apparatus, in which the carbon dioxide was absorbed while the oxygen percentage was allowed to fall. As soon as cyanosis began to appear hyperemia of the ears developed and became very much pronounced as the oxygen content of the blood was diminished. In a case like this, organic acids are undoubtedly produced and will be present in the blood, perhaps in considerable quantities. They will not, however, be able to raise the hydrogen ion tension of the blood to any appreciable extent, since they will liberate  $\text{CO}_2$ , which will be eliminated through the lungs. The experiments of the British Committee on Shock (Medical Research Committee, No. 25, p. 272), among others, clearly show that very large amounts of acid can be introduced into the blood of a living animal without producing any perceptible change in hydrogen ion concentration, so long as the respiratory center maintains its activity. In our case, the  $\text{CO}_2$  percentage of the expired air remained low (3.00 per cent when the  $\text{O}_2$  had fallen to 7.17 per cent and the hyperemia was marked), so that it can be definitely asserted that the hydrogen ion concentration of the blood cannot have been responsible for the hyperemia, which must be associated in some other way with the lack of oxygen.

It follows from the rates of inflow measured by Lewis on the human arm that other tissues besides the skin must take part in the reactive hyperemia and there can be no doubt that the muscles must be involved perhaps even more than the skin (Goldblatt, 1926). Inflow rates increased from 3-4 to 55 cc. per 100 cc. tissue per minute are possible only when the muscles participate to a large extent. A single experiment

has been made by Rehberg and myself on a cat in which we watched the circulation in the abdominal muscles. When oxygen lack was induced a large number of capillaries were opened up to close again when the animal was allowed to breathe air.

A reactive hyperemia cannot, however, be elicited in all mammalian organs. Bier (1897) has made occlusion experiments on the small and large intestine of rabbits and dogs and found no trace of reactive hyperemia in the empty intestines, while the processes of digestion would release substances producing some dilatation of the small vessels. In the stomach of rabbits the reaction was likewise missed, while it was present in the stomach of dogs. Bier's experiments on kidneys point to the absence in them of any reactive hyperemia, though the evidence does not seem quite clear.

To explain the phenomena of reactive hyperemia quite a number of theories have been proposed and it will be necessary to deal with these *seriatim*.

In the last century it was very generally assumed that the reaction often observed clinically, after occlusion of the circulation in the limb of a patient, was due to paralysis of the vasomotor nerves. Bier showed that this could not be the case by demonstrating that the reaction is obtained equally well if all the nerves to a limb have been severed previously. Grant and Lewis have shown that the reactive hyperemia is also independent of local nervous reflexes, for it occurs equally well on skin, the nerves of which have completely degenerated. Bier's conclusion holds also for the hyperemia due to lack of oxygen. In some experiments on the rabbit's ears Rehberg and I cut the sympathetic on one side in the neck and cut further the anterior and posterior nerves to the ear itself. No perceptible difference could be observed between the hyperemia in the two ears as a result of lack of oxygen.

We have some reason to believe, however, from a later experiment on the same ear, that the sympathetic innervation had not been completely abolished.<sup>4</sup> In another experiment of the same kind the arterial hyperemia on the operated side was definitely less pronounced. Both arteries and capillaries remained comparatively narrow, but a large number of hitherto closed capillaries were opened up, so that we must conclude that an essential part, at least, of the capillary dilatation was due to a peripheral effect of the oxygen lack.

To explain the reactive hyperemia Bier had recourse to the old conception of "Blutgefühl" mentioned in Lecture II as the attraction toward blood exerted by the tissues. Bier contended that the hyperemia can only be brought about by arterial blood to which the vessels open up while they react by contraction toward venous blood. The ingenious and suggestive experiments upon which this theory was based have been discussed before (Krogh, 1922) and it is sufficient here to show that the vessels relax during and not after the occlusion or lack of oxygen, and that they cannot be brought to contraction by venous blood.

On the rabbit's ear Rehberg and I have made the following experiment:

An area of about 1 sq. cm. in the ear is compressed by means of a Roy and Brown chamber until it is completely anemic. When the pressure is reduced after several minutes the field becomes very hyperemic. A clamp is arranged near the base of the ear so as to cut off the supply of blood. The blood becomes venous in color but the hyperemia of the area is maintained.

The area is compressed for ten minutes, the ear is clamped and the pressure thereupon reduced. In spite of the very low blood pressure resulting from the clamping, the blood pours into the area, which becomes

intensely hyperemic and remains so when the blood becomes venous.

Finally, the area is first compressed for fifteen minutes. A clamp is put on and kept on for fifteen minutes, during which time the blood in the clamped ear becomes very venous and the capillaries dilate. After the thirty minutes the area is decompressed, with the result that the venous blood flows in slowly from all sides and fills the capillaries, which cannot become much dilated, however, because the supply of blood is insufficient. Opening the clamp for a moment produces an intense hyperemia.

Lewis has made a corresponding experiment with quite similar results on the human arm.

The inrush of blood with the first pulse after occlusion is conclusive evidence that the arterioles, at least, were relaxed beforehand, and Lewis quotes an experiment by Kendrew showing the same for the vessels generally by recording a continuous fall in venous pressure during occlusion. Contraction of the relaxed vessels begins very soon after the release of the circulation or oxygenation of the blood.

Bayliss (1902) who studied the reaction in animals came to the conclusion that the arterioles increase in tone as a response to raised pressure within them and decrease in tone when their arterial pressure is lowered. Though this may be, and probably is, a factor in the response to arterial occlusion it can have nothing to do with the hyperemia following venous congestion or oxygen lack, and even after occlusion it can only contribute slightly to the total reaction, since, as pointed out by Lewis, it cannot explain the relation between the duration of occlusion and the duration of the subsequent hyperemia.

Lewis and Grant (p. 179) have investigated the possibility that lack of pituitary hormone, which is prob-



ably destroyed during occlusion, might be directly responsible for the subsequent hyperemia. By injecting 1/20 or 1/30 cc. intravenously they obtained a conspicuous blanching of the skin, but arrest of the circulation to a limb in subjects so treated was followed by a vivid hyperemia, seemingly as intense as in control observations. Owing to the excess of pituitary substance in the circulating blood the hyperemia faded more quickly, however. In another experiment one arm was first completely occluded. Immediately afterward the pituitrine was injected and, when after 20-30 seconds the subject's face was conspicuously blanched, the other arm, which now contained an excess of pituitrine, was also occluded. When released simultaneously seven minutes after the injection both arms flushed equally, but again the one which had an excess of pituitrine faded more quickly. It is evident, therefore, that lack of pituitary hormone cannot be responsible for the reactive hyperemia after occlusion, which can at most be somewhat intensified, when the hormone is used up. When a reactive hyperemia is brought about by oxygen lack, while the circulation is intact the hormone must be present in normal concentration throughout, without interfering with the reaction. Having excluded the possibilities so far discussed Lewis (p. 181) turns to the alternative that we have to do with a vasodilator substance. This is made extremely probable by the long duration of the reaction after a prolonged occlusion, and from the same fact Lewis concludes that the vasodilator substance must be outside the vessels in the tissue spaces, because when in the vessels it would rapidly be washed away. Lewis argues further that it must be a normal metabolite and identical with his H-substance and as this point is very important it is necessary to consider the evidence in some detail.

A very ingenious experiment *appears* to show conclusively that we have to do with a substance of low diffusibility collecting in tissue spaces but not rapidly taken up by the blood. I quote this experiment (p. 182) in full:

An Esmarch bandage is wound spirally around the hand and forearm depleting it of blood up to a pneumatic cuff (1 in Fig. 67), the pressure in which is now raised to a point far above systolic pressure. The bandage is removed and the blanched forearm and hand exposed; the empty veins are to

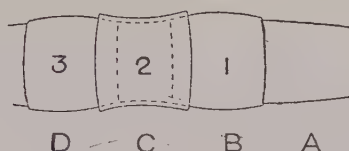


Fig. 67. Transference of blood.  
After Lewis.

act as a reservoir. When 10 minutes have elapsed, the pressure in cuff 1 is reduced until it lies just above the known systolic pressure of the subject, who now by briefly and forcibly expiring, forces a single jet of blood through the cuff. Little practice is required to drive an isolated pulse beat through the cuff. The effort is repeated every 10 seconds, and thus a very slow percolation of blood through the capillaries is obtained. The slow percolation of blood through the vessels favors diffusion of the supposed vasodilator substance, accumulated in the tissue spaces during the 10 minutes' arrest, into this blood to an unusually high concentration; the blood collects in the empty veins. When a sufficiency has been caught up, and this happens in the space of about 5 minutes, this blood is transferred to a new vascular territory.

The transference is effected as follows: Another cuff (3) is placed high on the upper arm; a third cuff (2) completely overlaps the edges of cuffs 1 and 3 and covers the portion of skin that lies between them. Cuff 2, and immediately afterward cuff 3, is forced to 200 mm. pressure and cuffs 1 and 2

are at once removed. The skin so exposed (areas B and C) contains little or no blood, area A is turgid with stagnant blood. The arm is held up enough to allow this blood, which it is desired to test, to percolate into the skin of area C. The skin of this area suffuses quickly and deeply; it is allowed to remain so for varying periods up to 5 minutes, when the last cuff, 3, is removed. Now the skin of regions C and D has been treated as follows:—each has been depleted of blood and deprived of circulation for say 5 minutes, each, therefore, will as a result display hyperemia on release; but into C blood from A has been introduced for the greater part of the time. If the transferred blood contains vasodilator substances, their effect should be added and the hyperemia on release should be more vivid and lasting in area C than in area D. In point of fact this is not the case, the hyperemia that appears is equal over C and D and lasts no longer in the former than in the latter. Meanwhile areas A and B, so long deprived of circulation, experience equally the usual vivid and long-lasting hyperemia. It seems clear, therefore, that there can be no appreciable concentration of substances in blood passing even very slowly through tissues previously long deprived of circulation.

I cannot accept this conclusion as proving that the substance must be of low diffusibility. If, on the contrary, we suppose the substance to be highly diffusible, its concentration in the transferred blood cannot exceed that in the tissues of area A and the quantity available in the capillaries of area C is, therefore, probably insufficient to prolong the hyperemia.

From the fact that a distinct reactive hyperemia could be obtained by a few seconds' occlusion Bayliss concluded that it could scarcely be due to any accumulation of metabolites, but Lewis controverts this view and says that "since there is a continued and steady increase in the reaction as occlusion is prolonged it is hardly to be doubted that the shortest and longest reactions are fundamentally alike." He refers also to

the brain as an organ, where deprivation of blood supply for five seconds disturbs the function so profoundly that unconsciousness supervenes,—“a loss of function difficult to explain except on a metabolic basis.”

Granted that the substance is a metabolite, we are forced, according to Lewis, to assume that it is a normal metabolite “for, as we have seen, a perceptible reaction can be shown in the limb to which the circulation has been arrested for so brief a period as five seconds. It is scarcely conceivable that the character of tissue metabolism alters in such a short period of time; it is readily conceivable that normal metabolites continue to form, but are not removed at a normal rate.”

An increase in temperature of the tissues increases the reactive hyperemia as beautifully shown in Fig. 68 and this “suggests that the dilatation is dependent upon the rate at which metabolism occurs in the tissues.” Having shown that heat in itself causes dilatation of the minute vessels by liberation of a vasodilator substance, and that there is a complete gradation from the slight reddening caused by moderate warmth to the full triple response, Lewis concludes that we have to do in all cases with the same normal metabolite and rounds up the evidence by showing that a prolonged deprivation of circulation (8 hours) over an area of skin leads to subsequent whealing, while a surrounding flare may become distinctly visible and a diffusion flush is often prominent.

Although the evidence brought forward by Lewis, and here briefly recapitulated, appears overwhelming I am unable to accept it as conclusive. The main reason for this is that it fails to explain the hyperemia found to accompany anoxemia. In the respiration experiments with observations on the rabbit's ear we have a free flow of blood to carry away the products of me-

tabolism, but in spite of that an intense hyperemia supervenes. The anoxemic hyperemia might have a mechanism different from the occlusion or congestive hyperemias, were it not that lack of oxygen is a factor common to all these forms of deprivation of blood.

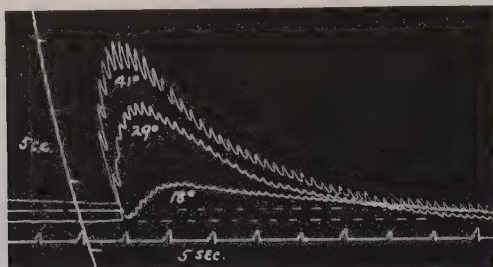


Fig. 68. Three curves of forearm volume, following the release of the circulation in each case after a 2-minute period of arrest. The curves were obtained after the limb had been for half an hour at one of the corresponding temperatures, namely, 18°, 29° and 41° C., respectively; and the records show the increasing reaction with higher temperatures. It is to be remarked that the base lines of the three curves do not really correspond, they have been brought together so that the differences in reaction may be shown more clearly; with a higher temperature the vessels of the limb contain more blood at the instant of arrest. After Lewis.

When there is lack of oxygen the metabolic processes are certainly not completely normal, and an oxygen debt is incurred which can be discharged afterward. When the brain, deprived of blood for five seconds, responds by unconsciousness, as cited by Lewis against Bayliss, this is practically certainly a case of anoxemia, since breathing of nitrogen for a few seconds will have just that effect. In resting muscles deprivation of circulation for five seconds will also according



to calculations to be given in a later lecture cause a definite lack of oxygen and the consequent formation of intermediary products of metabolism.

The evidence purporting to show that the substance responsible for reactive hyperemia is of low diffusibility cannot, as stated above, be taken as conclusive, and it is in direct conflict with the contention that we have to do with a normal metabolite. If "blood passing even very slowly through tissues previously long deprived of circulation" does not acquire any "appreciable concentration" of the substance there must be normally a very much higher concentration in the tissue spaces than in the blood, a concentration, in fact, corresponding to the production during many minutes, and it is inconceivable that five seconds' occlusion should raise this concentration sufficiently to produce a reaction which was not produced by the normal concentration. We must assume, therefore, that either the substance is of high diffusibility or else that it is not normal, but is being produced in those places where the occlusion results in oxygen lack.

In Lewis' experiment on the effect of temperature on the reactive hyperemia quoted above (Fig. 68) it is a very significant fact that the duration of the hyperemia is practically the same at all temperatures. Lewis explains this by a reference to the increase in blood flow at the higher temperatures, but the removal of a substance of low diffusibility will not depend mainly upon the blood flow, but upon the rate of diffusion which is very slightly affected by temperature. Again it appears necessary to conclude either that the substance is of high diffusibility or else that it is not normal. Lack of oxygen at a high temperature will certainly entail an increased production of intermediary metabolites which after the admittance of blood are partly carried away, but probably to a considerable

extent oxidized *in situ*. The rate of oxidation is accelerated by high temperatures in such a way that it is easily conceivable that the duration of the hyperemia is about the same at different temperatures.

The final argument that a prolonged deprivation of blood brings out the full "triple response" is certainly the most formidable—if we accept the reasoning that a perfect gradation of response from a fugitive reddening through whealing, blistering, and pigmentation means that only one substance is at work in increasing concentration and duration of activity. In speaking of the reaction to light I have given reasons for the conclusion that there must be at least two H-substances. I find no difficulty in the idea that cells deprived of oxygen for eight hours are damaged to such an extent that colloid vasodilator substances are liberated which are only slowly removed through the lymph vessels, while the normal phenomena of reactive hyperemia are due to comparatively simple products of a metabolism going on under an insufficient supply of oxygen.

Summarizing the whole of our discussion of indirect reactions I accept unreservedly the main thesis of Lewis that the tissue cells will under a number of stimuli liberate substances having a dilator action on their smallest blood vessels, but I find it impossible to assume that we have in all cases to do with one and the same H-substance and to take this to be a wholly normal metabolite regularly discharged into the blood stream. Even if we limit the conception to the skin I cannot accept as conclusive the evidence for the view that such a substance is regularly discharged. As I take it a vasodilator substance is liberated whenever there is oxygen lack, certainly in the skin, very probably in muscles, perhaps in other tissues, but not in the intestine of mammals. This substance is responsible for all the reactions following upon occlusion of the blood

supply or upon congestion, when this is sufficient so to reduce the blood supply that lack of oxygen occurs in any part of the tissue. I hold it to be responsible also for the reaction to increased temperature which causes oxygen to be used up at more rapid rate, and it may also, possibly, cause the opening up and dilatation of muscle capillaries during work. This substance may be histamine, but the evidence for the identity is slight only, and the absence of the characteristic sensation usually produced by histamine as well as the apparent absence of any action on sensory nerves producing a "reflex" flare makes it rather improbable, in my opinion, that it is histamine. The absence of a reaction to occlusion from the intestine, which reacts normally to histamine, also militates against the identity.

Whenever cells are damaged in mammalian skin, and probably also in other organs in mammals, histamine—or an H-substance closely related in its physiological activity to histamine—is liberated and acts on the vessels. The amount of histamine set free by a given stimulus is evidently very variable and the large variations in susceptibility of individuals and species to different forms of tissue injury are largely to be explained as variations in histamine production and only to a minor, but unknown, extent as variations in reactions to histamine. It should be remembered, however, that in the frog, where the reactions to injury are very pronounced, histamine fails absolutely to produce any response. The mechanism, which is probably essentially the same, must, therefore, in this case involve the liberation of a totally different substance.

According to the experiments of Lewis referred to in the sixth lecture the H-substance (histamine) not only stimulates sensory nerve endings, but is produced by them when stimulated. We have to assume, therefore, that in the reflex flare in human skin H-substance

is liberated over the whole of the area involved in the flare in sufficient quantity to cause some relaxation of arterioles, capillaries, and venules. In this case again we have to assume that, though the mechanism may be the same for all mammals or indeed for all warm-blooded animals, the substance liberated after stimulation of sensory nerves in frogs must be totally different.

In certain reactions and notably in the slow response to light a substance of very low diffusibility, probably colloidal, appears to play a prominent part. This substance does not, apparently, stimulate sensory nerve endings. No pain is evoked and, what is even more important, no flare is produced. In my opinion the flare is the normal response to a long-continued stimulation of the endings of pain fibers, and the absence of a flare in certain slow reactions points to the conclusion that the diffusible H-substance (histamine) never reaches a perceptible concentration in the tissue spaces, but is carried away by the blood as rapidly as it is formed, while the indiffusible (H-colloid) is accumulated.

While the difference between direct and indirect actions on capillaries through the medium of tissue cells is undoubtedly fundamental, there are border line cases, in which more than one kind of stimulation takes place, and I cannot but agree with Heubner (1925) that certain substances and stimuli act both on tissue cells and on nerves or capillaries or both.

Mustard oil is one such substance which according to Lewis produces a flare out of proportion to its local action, while the experiments and observations of Breslauer show that it loses practically all its activity when applied externally to skin, the sensory nerves of which have degenerated. On the conjunctiva the action is partly direct (Hirschfelder, 1924). Galvanic stimu-



lation probably also affects the pain organs directly, but the most beautiful example of an axon reflex flare produced directly by nerve stimulation before any liberation of H-substance could take place in the directly affected area is given by Lewis as the reaction to freezing of the skin. A reflex flare appears 35 to 45 seconds after freezing has begun, even when the application of cold is continued, and this flare may fade during thawing to reappear later when the damaged cells begin to pour out H-substance. In the frozen condition an outpouring of H-substance seems very unlikely.

### NOTES

<sup>1</sup> Among the substances giving the triple response when pricked into the human skin are the split products of proteins—albumoses and peptones. Lewis has tested (p. 63) a sample of Fairchild's peptone (concentration 1 in 10) and fractions of this substance prepared by dialysis and by precipitation with alcohol and ether, and found all fractions, including the alcohol insoluble and the ether soluble, active, so that the activity can scarcely depend upon the histamine content of the original preparation. In Abel's laboratory (Abel and Geiling, 1924; Geiling and Kolls, 1924) purified primary albumoses, prepared from peptone and containing no histamine, have been tested by intravenous injection into dogs in quantities of 200 mg. per kg. They produce considerable capillary dilatation in the skin and visible mucous membranes, and intestinal congestion. The dilatation is not immediate like that of histamine, it lasts for an hour or more, and after repeated injections even at 24 hours' interval the symptoms become much reduced, while a few dogs are naturally insusceptible to albumoses. In dogs anesthetized with ether the reactions are either absent or much reduced.

All these facts are consistent with the hypothesis that protein split products produce their dilator action on capillaries through injury to tissue cells which in their turn liberate the H-substance, but there is the other possibility that the albumoses themselves are the source of origin of the vasodilator substance.

<sup>2</sup> Török (1928, p. 66) describes experiments pointing to an immediate production on irradiation of an active substance which is taken up by the blood. He finds on irradiating a large part of the human skin that the reaction to histamine and H-substance is increased and further that the blood from irradiated subjects will itself produce increased reaction on intracutaneous injection. This power seems to disappear after the irradiation to reappear when the visible local reaction sets in. In these experiments the active substance must be one of very high diffusibility.



<sup>3</sup> My attention has been called by Dr. Haxthausen to the haemolytic action of ultraviolet light which shows a very striking analogy to the reactions of the human skin. When blood corpuscles suspended in agar are suitably exposed a haemolysis will take place after a few hours and be strictly limited to the exposed area, but after 1-2 days the haemolysis spreads a little toward all sides. I agree with Dr. Haxthausen in thinking it very likely that a special substance of very low diffusibility is formed photochemically, perhaps from lipoids, both in vitro and in vivo and that this gives rise to the liberation of H-substance by damaging the cells. If the formation of this hypothetic substance takes place mainly in the deeper layers of the epidermis the long latency of the light reactions would be explained.

<sup>4</sup> Fletcher (1898) has shown that the sympathetic fibers to the rabbit's ear do not all pass through the cervical sympathetic. Several of them pass from the stellate ganglion along the ramus vertebralis on to the third cervical nerve and thence to the ear.

## LECTURE XI

### COMPLEX CAPILLARY REACTIONS AND THEIR SIGNIFICANCE

**W**E have still to consider a small number of peculiar and complicated reactions of skin capillaries, the mechanisms of which are more or less obscure, but must be mainly indirect. Several of these have been observed so far only in the human skin.

#### *Paleness of death.*

I have described in a preceding lecture the effects on the circulation in the rabbit's ear when the animal breathes air with a low and declining oxygen percentage. It was shown that along with the cyanosis a very pronounced capillary hyperemia of the ear resulted. A moment comes, however, when the hyperemia is suddenly reversed, the arteries, capillaries, and venules contract very strongly and the ear, which was, just before, extremely hyperemic and deep blue, becomes strongly anemic and pale, both macroscopically and microscopically. It is an extremely striking observation when the ear is watched under the microscope to see all the small blood vessels contract and pour the blood into the veins. The macroscopic veins themselves are not visibly affected. This change comes, generally, when the pulse has become irregular and the respiration is reduced to single spasms at comparatively long intervals.

This change corresponds to an observation by Bier

(1897, No. 27) who produced a reactive hyperemia by prolonged occlusion of the hind leg of a pig. During the hyperemia the animal was asphyxiated, and when the skin became blue the hyperemia quickly faded away to develop again when the animal was allowed to breathe.

Hooker (1920) made quite similar observations both microscopically and macroscopically on the ears of cats that were suddenly killed by an overdose of ether, and it is possible, therefore, that asphyxia is not a necessary condition for the development of a premortal contraction of the smallest blood vessels.

It is evident that this reaction is responsible for the well-known "paleness of death," which may often be so conspicuous, also in human beings. It may be absent in certain cases of disease, and this has its parallel in Hooker's observation that in cats in a state of histamine shock the capillaries remained dilated at death.

To find the mechanism of this striking reaction Rehberg and I have, in several experiments, cut the nerves to one of the ears before the respiration experiment. Cutting the anterior and posterior auricular nerves, which are mainly sensory, has no effect whatever, but section of the cervical sympathetic either abolishes the contraction reaction altogether or diminishes its intensity to a very marked extent. The latter result is probably due to the sympathetic fibers reaching the ear by way of the third cervical nerve (Fletcher, 1898). In any case the observed contraction is due to a powerful stimulus sent out, probably from bulbar centers, along sympathetic fibers.

In his experiments on the cat Hooker observed that section of the cervical sympathetic did not prevent the reaction in the corresponding ear, and we must conclude, therefore, with Hooker that in this case the cen-

tral nervous system is not involved, but the contraction must be brought about by some local mechanism. The existence of such a mechanism in man would help to explain the phenomenon known as "Bier's white spots."

In his experiment 76 (1898), on the arm of a fair-skinned man, Bier produces a slight hyperemia by venous stasis, so that the arm is slightly blue, and then completely occludes the blood supply with an Esmarch bandage. The arm is allowed to hang down before a hot air register. At first it is a uniform blue, but soon becomes spotted with white, and after fifteen minutes the white spots predominate on the upper arm. At this time the forearm and hand are still largely blue, but white spots do not fail to appear even on the finger tips. An examination of the blue areas with a hand lens shows that there are many little white islands in them.

The white spots discovered by Bier were studied in my laboratory by Rehberg and Miss Carrier. The spots show best, owing to the greater contrast of color, when a hyperemia is produced before a pressure cuff is applied to shut off the supply of blood. In this case white spots begin to show on the blue arm after five to ten minutes. They grow in size until they are one or more centimeters in diameter or merge into larger patches, covering a considerable portion of the arm. Previous to these spots on the arm there also appear light red spots, the color of arterial blood, on the hand, where true white spots are seen only occasionally.

Microscopic observation of the back of the hand in an area remaining blue while the flow is stopped shows the following changes. During the first minute or two the open capillaries shift, as they do normally, but gradually more and more become visible in the field, until, at the end of two to three minutes, all the capillaries are open and remain so for the twenty to twenty-five minutes' duration of the experiment. The underly-

ing vessels, however, are only just visible. The blood becomes a deep blue color. On releasing, the color almost instantly changes to arterial, the background becomes red also and the venous plexus very full and prominent. This is quite transitory, however, and in a minute some of the capillaries begin to disappear, the background is less brilliant, and two or three minutes later the skin looks quite normal. If a red spot during occlusion is observed under the microscope it cannot be distinguished from a field with a normal circulation. A white spot on the back of the hand shows microscopically a complete disappearance of underlying vessels and the tips of a very few capillaries only are visible. When the pressure is released the white spot also becomes hyperemic but not so intensely as the surrounding blue skin, and it returns even more rapidly to a normal condition.

In the arm a definite distinction between "red spots" and "white spots" could not be arrived at by means of the microscope, because the normal color of the skin is white and only a few capillaries were normally open. The evidence presented, however, justifies the conclusion that there are two kinds of spots. The first are arterial spots, that is, areas containing arterial blood, fed probably through the collateral circulation by the medullary branch of the superior profunda artery, which runs for a distance through the humerus, so as not to be compressed by the cuff, and then sends a branch to anastomose with the recurrent radial. It was observed that if the finger was pressed over such a spot, so that the blood was pressed out of it and the surrounding tissue, when the pressure was removed the blood leaked into the surrounding tissue from the periphery, but the arterial spot itself filled immediately from below. It is a rather remarkable fact that the small supply of arterial blood which gets into



the occluded arm through anastomoses in the bone can be located in definite spots on the surface, usually to be found again and again in the same places.

Rehberg and Miss Carrier found that the true white spots would become larger and more numerous when the arm was cooled and concluded that their appearance was due to the cooling which is inevitable when the blood supply has been cut off, but the observations of Miss Wolf (1924, Lewis, p. 280) show that this explanation is insufficient. In the first place it does not account for the fact that the contraction of the minute vessels is limited to certain areas, while the fall in temperature affects the whole surface practically uniformly, but Lewis has found further that true white spots may appear in an occluded arm immersed in water at 41° C., while the venules in other areas dilate. Lewis has observed that Bier's white spots appear also on insensitive skin, the nerves of which have long since degenerated, and concludes that nervous influences and especially axon reflexes cannot be responsible. When this is so it seems difficult not to accept the hypothesis into which Lewis summarizes his very instructive discussion concerning the white spots, namely, that vasoconstrictor substances are produced along with the vasodilator substances formerly discussed when the blood supply has been cut off for some time. It is shown to be a necessary consequence that the former should be considerably more diffusible than the latter.

*Capillary reactions in the economy of the organism.*

We have dealt up to this point mainly with the mechanism of the reactions studied, and while we have found that some of them can be fairly definitely accounted for, we have to admit that the mechanism of others is very obscure. We come now to consider the part played by capillary reactions in the general proc-

esses of regulation and defense of the organism. We shall meet again some of the reactions already studied, but now approached from a new point of view, and we shall discuss others, the significance of which for the organism is evident, while their mechanisms are entirely unknown. Reference will be made finally to reactions which are obscure from every point of view and to certain pathological conditions.

In the regulation of normal blood flow we have to distinguish, as already emphasized, between arteriomotor and capillariomotor control, regulating respectively the volume flow and the conditions of exchange between the tissue and the blood flowing through it. The exchange of substances will be discussed in detail in the next lecture, and only the general principles will be touched upon here.

By dilatation of arterioles the flow of blood per minute through a unit of tissue is increased. Even with a considerable increase the capillary bed may remain practically unaltered and we get only an increase in velocity. This is perfectly sufficient, e.g., for the dissipation of heat, and it also improves generally the conditions for exchange of material by increasing the average difference in concentration of diffusible substances. A considerable improvement of the conditions for exchange cannot, however, be obtained by a simple increase in flow, but requires processes of regulation within the capillary system itself. When capillaries dilate, the surface through which the exchange takes place is increased in proportion to the increase in capillary diameter, and when new capillaries are opened up to the flow between those formerly open, the distances through which substances must pass between tissue cells and the blood is materially diminished. In certain tissues only a fraction of the total number of capillaries are necessary to provide for the

needs of the cells during rest, while more are opened up during activity; in other cases all the capillaries appear to be normally open, while only their diameters are subject to regulation. Generally the number of open capillaries, even in a resting tissue, is sufficient to admit a considerable increase in blood flow brought about by dilatation of arterioles, which are normally very narrow.

The existence of a large number of closed capillaries in resting muscles of frogs and warm-blooded animals was discussed in Lecture III, where it was also pointed out that the open capillaries were found to be fairly regularly distributed throughout the muscular tissue.

Large numbers of capillaries are also normally closed in the tongue, bladder, and intestine of frogs.

In the frog's kidney Richards (1922, Richards and Schmidt, 1925) has observed that with a slight diuresis many glomeruli are completely cut off from the blood stream and may either appear empty or contain a number of stagnant corpuscles. In the single glomerulus several of the capillary loops are also usually closed to the flow, although they appear to be contractile only where they spring from the afferent arteriole.

In the frog's skin, brain, lung, and liver practically all capillaries normally appear to be open, while the current through them is very variable. In the skin they often contract so as to make the passage of corpuscles difficult, but rarely so as to stop the flow altogether.

On mammalian organs the observations made are not very numerous and on some points conflicting. There can be no doubt with regard to the conjunctiva in which most of the capillaries are normally closed. In the skin of the ears of several mammals—dogs (Kolls and Geiling, 1924), cats (Hooker, 1920), and rabbits (Krogh and Rehberg)—it is easy to observe that a considerable number of capillaries are normally closed.

With regard to human skin the evidence appears to be somewhat contradictory, and the state of the small vessels seems to vary not only from one cutaneous region to another, but to be different in different individuals. It seems to be agreed, although the evidence is not quite sufficient, that the venules, which act as giant capillaries and constitute a fairly regular net parallel to the surface, below the papillae, are always open, but vary greatly in bore. The capillary loops are practically always open at the base of the nails, where they can be most easily observed, though occasionally single loops disappear from view. Lewis (pp. 197-198) has found on a number of subjects that the capillaries on the back of the hand, wrist, and arm are also practically all open in ordinary conditions, while Miss Carrier (1922) observed on a few subjects that only a variable number (between 15 and 50 per cent) of capillaries were simultaneously open on the back of the hand. The observations of Weil (1924, p. 196) and those of Brown and Sheard (1926) referred to below (p. 256) agree in the main with Miss Carrier's, while L. Fischer (1927) confirming the opening and closing of single loops has found on an average about 70 per cent open capillaries at any one time.

Rehberg and I have now made some supplementary observations, and we can confirm the statements both of Lewis and Miss Carrier. In some subjects and in some parts of the skin of our subjects the capillaries are habitually open though the visibility of single loops is rather variable. In others, and notably in the protected skin of the forearm of one subject (*V.L.*), we find normally only a small number of open capillaries, while many more are opened up by gentle stroking performed with a blunt glass rod under the microscope. In the forearms of three children which were constantly exposed and pigmented practically all cap-



illaries were open. I suspect that differences in the rate of growth of the epidermis may be responsible for this discrepancy.

In the white tissue of scars a large number of capillaries would appear to be closed, though a microscopic study has not been made. The capillaries are there, but they react differently, according to Ebbecke (1917) from those of normal skin, giving neither the white nor the red local reactions. When exposed to cold, they relax at an earlier stage and make the scars stand out with a distinct blue color against the otherwise pale skin (Ebbecke). Bier (1897, p. 452) mentions the case of a very seasick man: his face was deathly pale, but the numerous scars from his student duels stood out with a blue color. We can infer that the capillaries and venules in the scars had failed to contract along with the arteries. The dilatation may have been a consequence of the oxygen lack.

Otfr. Müller (1922, p. 33) quotes the observations of Hueter (1879) who found a large number of capillaries on the inner surface of the lower lip of man to be normally closed and to become opened by stimulation.

Sternberg (1927) has shown by vital injections on guinea pigs that the mucous membrane of the nose and upper respiratory passages with the exception of the vocal cords have numerous open capillaries while most are closed in the smaller bronchi. When a tracheal cannula is inserted most of the capillaries which are no longer exposed to the air currents become closed.

As referred to above Rich (1921) has studied the capillaries of the omentum in cats. Practically every fat cell is supplied with a capillary loop, but in the normal resting condition only a fraction of these loops are patent, while the slight stimulus of exposing the omentum to the atmosphere at  $37^{\circ}$  is sufficient to pro-



duce a general hyperemia, in which all the capillaries are opened.

Hayman and Starr (1925) have used injection methods on the mammalian kidney and found that when diuresis was not very active only about one-half of the glomeruli were open, but no attempts were made to study individual capillaries within the glomerulus.

Studying the bone marrow of pigeons by regular injections Doan (1922) found on starved animals a large number of capillaries which "appear to have been non-patent and functionally dormant so far as circulation is concerned. They are collapsed so that only a trace of fine ink granules reveals the presence of a potential lumen, the caliber of which appears insufficient for the passage of even a single blood-cell element without difficulty. In an ordinary injection (on non-starved animals) they are totally collapsed and are seen as septa surrounding the fat cell spaces." Drinker, Drinker, and Lund (1922) describe a similar arrangement for the mammalian bone marrow, but have not obtained such clear evidence, because they have only injected normal animals.

In the lungs Toyama (1925) has found by vital injections of lithium carmine that during rest a large number of capillaries were "reserved," while during work all were taking an active part in the circulation, and Wearn, Barr, and German (1926) report corresponding microscopic observations on the living and completely intact lung, which they have succeeded in studying without opening the chest. In spite of this I have some doubt whether lung capillaries are ever actively closed. Hall (1925) has found no evidence by direct microscopic study of closed capillaries in the lungs of cats and rabbits, but his procedure for exposing the lung was scarcely sufficiently delicate.

The biological significance of the fact that a large

number of capillaries in certain organs, of which the muscles are by far the most important, are normally closed, is the economy attained with regard to the blood volume. The blood available in the organism is insufficient to fill the vascular system, when all capillaries are open so as to allow the passage of corpuscles. On the other hand a small number of open capillaries is in many cases sufficient to give the necessary surface and the corresponding diffusion distance to meet the needs of a resting tissue.

It will be shown in the succeeding lecture that in such a system, with a just sufficient number of open capillaries a relatively long distance apart, the distribution of the substances supplied by them will be very unequal. The tissue elements nearest to the open capillaries will get more than they require, while the more distant will be comparatively starved. I have on this basis developed the idea that the position of the open capillaries may be continuously changing so that a cell, starving at one moment, will get all it wants when a capillary near to it is opened up. In this way an adequate, though intermittent, supply can be secured at every single point, while at the same time, the blood is utilized with the utmost economy, the necessary minimum only being present at any one time in any tissue.

It must be admitted that the evidence at present available for such a shifting of open capillaries is absolutely inadequate to show that it is a phenomenon of general significance in the economy of the organism and scarcely sufficient to show that it exists at all. Observations of this kind are particularly difficult to make and record, and really objective evidence can, I believe, only be obtained from micro-cinematographic records. The technical obstacles met with in the attempt to secure such records are formidable, but renewed attempts will be made to get round them.

Visually I have seen indications of such shifting in frog's muscle, when keeping a small field with a few open capillaries under observation for 15 minutes or more.

W. Hagen (1921) has seen a series of changes in the flat capillary network in the rabbit's ear and gives a diagrammatic representation of shifting during a few minutes. Miss Carrier (1922) has observed shifting of capillary loops in several fields in the back of her own hand and gives the adjoined figures (69 and 70) of a single field.<sup>1</sup>

Richards and Schmidt (1925) describe shifting of the blood stream within the capillary tuft of the frog's glomerulus where it is brought about apparently by the action of contractile elements at the entrance to each capillary. Of even greater importance, if only by analogy applicable to capillary conditions, is their observation which has been often repeated and confirmed, that in the resting frog's kidney, where, as we have seen, only a certain number of glomeruli are open, there is a continuous opening and closing of glomeruli so that, as Richards and Schmidt suggest, no single glomerulus is deprived of blood long enough to cause damage to the endothelium.

The mechanism of the shifting of capillaries when the supply of blood is insufficient or barely sufficient to supply the needs of the tissue is not known. It can be conceived as due to an interaction between vasodilator substances—Lewis H-substance—produced, for instance, where the supply of oxygen fails and the pituitary (or other) hormone which will cause an increase in capillary tone when the current of blood through a capillary is ample. A continuation of the work of Lewis on suitable objects should be likely to furnish evidence on this point.

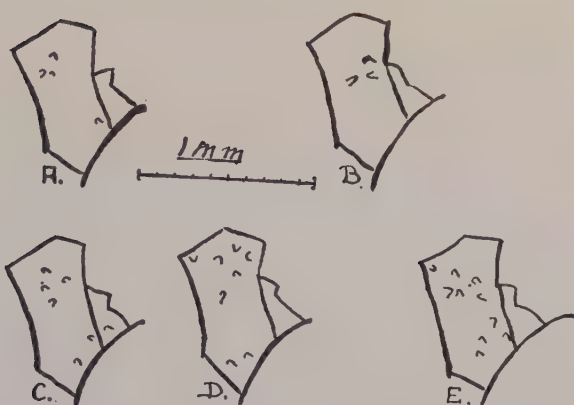


Fig. 69. Area from the back of the hand bounded by underlying vessels. Capillary tips visible on the 12th of December at 11 (A), 14th at 11 (B), 15th at 11 (C), 12 (D), and 4 P.M. (E). After Carrier.

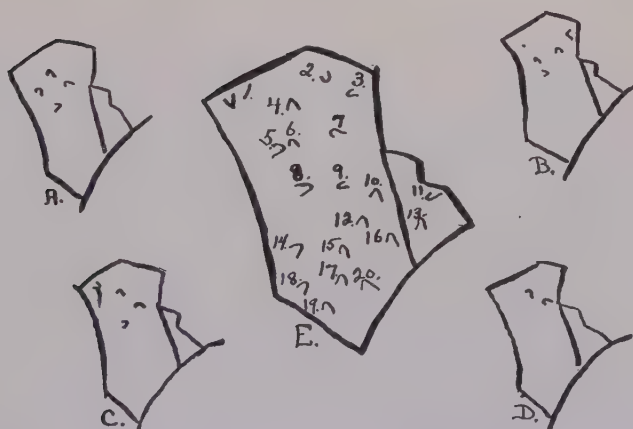


Fig. 70. The same field at 3-minute intervals. (A-E) at E after light pressure. After Carrier.

*Reactions to blood volume.*

The principle of adjustment to the supply of blood is clearly illustrated in the reactions to natural or artificial variations in the total available blood volume.

It is well known that a large loss of blood brings about a very characteristic paleness of the skin and mucous membranes. Though this paleness is probably partly due to the dilution of the blood which occurs rapidly after hemorrhage, it seems very likely that an active contraction of capillaries and venules may also be responsible for it.

In experiments in which dogs were bled to a very large extent Meek and Eyster (1921) observed a sudden contraction of the microscopic vessels of the ear, but their description points rather to a premortal contraction than to a compensatory reaction. They point out, also, that the quantity of blood in the skin is so small (2 to 3 per cent of the whole) that the contraction would have very little effect if it were confined to the cutaneous vessels.

The evidence for an opening up of skin capillaries and venules in plethora is more definite. Meek and Eyster (1922), who studied experimental plethora in dogs, showed by photographing the same field of ear vessels before and after the injections of gum saline that a considerable number of new capillaries were opened up and the venules dilated, and beautiful studies of a quantitative character have been made on man by Brown and Giffin (1926) and especially by Brown and Sheard (1926) who made skin color determinations, countings of capillaries, and determinations of capillary areas in cases of polycythemia vera, in which the total blood volume was considerably increased, and in normal controls. Studying by instantaneous photography corresponding areas on the fingers they observed an increase in the number of



open capillary loops in their patients. This increase, from an average of 40 per sq. mm. to 65, is not very large, but is undoubtedly outside the limits of error. The single loops were enlarged, and while their total cross section averaged 5 per cent of the surface in the controls 15 per cent was found in the patients. In two patients the blood volume was reduced, though apparently not brought down to the normal level, and in both the number of open capillaries went down to about one-half of that previously found, while the cross section of the single loops was comparatively slightly reduced.

These reactions to variations in the blood volume cannot be explained as simple effects of a reduced or increased pressure in the vessels concerned. The pressure variations, if any, are much too small for that. There must exist a special mechanism to bring them about, but regarding this only guesses can be made. The capillary reactions to blood volume changes appear to be rather slow. There are other regulating mechanisms capable of rapid action.<sup>2</sup>

#### *The response to activity.*

The vascular response to cellular activity is a dilatation of arterioles with the consequent increase in blood flow and generally also a dilatation and opening up of capillaries. This latter reaction is especially conspicuous in muscle, where even a single contraction never fails to bring about a distinct increase in the number of open capillaries. In the frog, stimulation of muscle nerves fails to cause any opening up of capillaries when contraction is prevented by curari, but a reflex relaxation of sympathetic tone is an obvious possibility in voluntary or reflex contractions. After the work of Lewis we would expect the reaction to be due to the local production of a vasodilator substance,

and the response is certainly worth studying from this point of view.

*The response to injury.*

While the biological significance of the activity response is evident and universal, the corresponding response to injury is from a teleological point of view more complicated. The responses which minimize loss of blood referred to above (p. 143) and studied in greater detail by Herzog (1925) are clearly beneficial, and there can be no doubt that both the increase in blood supply and the improved conditions for capillary exchange, following injury of any kind in skin and muscles and perhaps in other tissues, help to remove injurious substances introduced or produced by injured cells and to repair the tissue. It is not, however, possible to account satisfactorily for the significance of the single elements in the response. Lewis (p. 21) considers the "reflex flare" as a response mobilizing the defense of the "threatened points" surrounding the actual seat of injury. To my mind it seems very doubtful if in ordinary circumstances of the life both of man and other mammals the surroundings of an injured spot are more immediately threatened than the skin generally, and also somewhat doubtful if a general opening up of arterioles prior to an injury is really beneficial. It will almost certainly lead to increased loss of blood in case of the injury being mechanical, but the interesting experiments of Török, Lehner, and Urbán (1925, p. 391) seem to show that in the hyperemic zone the visible reaction to poisons is reduced.

The free exudation of plasma from the capillaries in injured skin may be beneficial in placing the plasma proteins at the disposal of the cells and may also help to wash out injurious substances or particles which are

unable to penetrate the capillary wall, but in many cases the reaction certainly exceeds the possibly beneficial level and becomes directly harmful. Lewis has found (p. 54) that arresting the circulation for fifteen minutes after bruises or severe freezing will diminish the subsequent whealing and expedite the rate at which the whole reaction subsides. It should be remembered also that on denervated skin mustard oil is a compara-

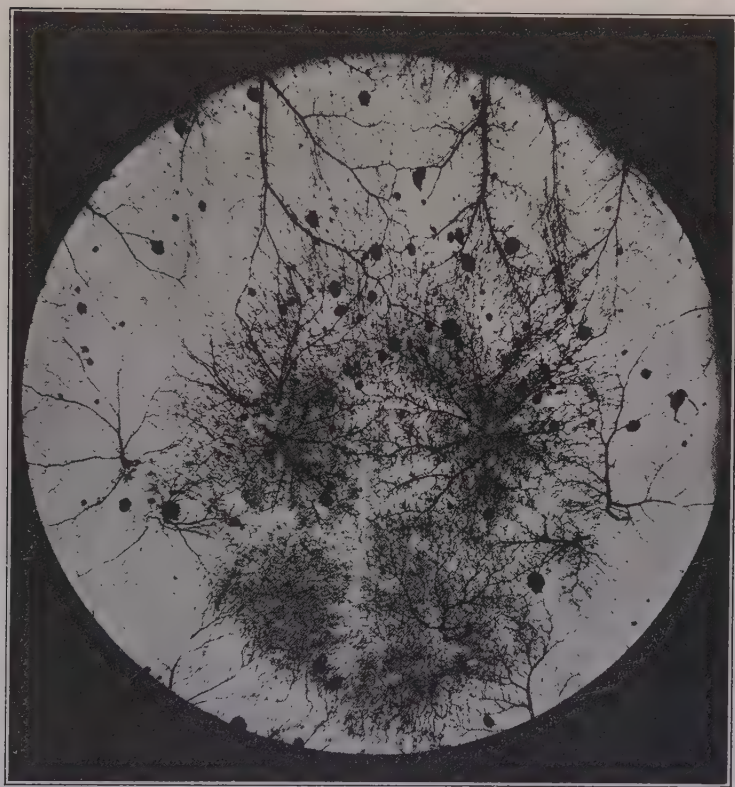


Fig. 71. Skin of mouse vitally injected with India ink after local treatment with tar (10 times in 22 days). Outside the tar painted area most of the capillaries are closed. After Kreyberg.

tively harmless substance, while normally it evokes a painful and probably somewhat harmful reaction.

When the skin of a mouse is treated with tar leading ultimately to the development of cancer, the first reaction has been found by Kreyberg (1927) to be a very considerable hyperemia involving all classes of vessels and well shown by the photographs of vital injections which Dr. Kreyberg has kindly placed at my disposal before publication (Fig. 71). It seems probable that if the skin failed to react in this way to the tar, very little harm would result.

It is, of course, only what one would expect that reactions which are essentially defensive in character cannot be equally adapted to all the forms of attack to which the organism can be exposed.

#### *Other responses.*

A very curious reaction has been described for the fish *Fundulus* by Connolly (1926). This fish shows a very pronounced color adaptation to its surroundings, and when in red surroundings the skin becomes red, the color is found to be only partly due to expansion of chromatophores, but to a large extent to dilatation of skin capillaries.

Gänsslen (1927) reports reactions of the human skin capillaries to the character of the diet. He describes how after 10 days' exclusive meat diet the skin capillaries in the chest, arm, lips, and nail fold become dilated, showing numerous aneurysms and small petechiae reminding of the initial symptoms of scurvy. A lacto-vegetarian diet is reported to reduce the capillaries slowly (in 20 to 30 days) and eventually make them abnormally narrow.

#### *The vasoneurotic constitution.*

I shall say, finally, a few words about the so-called vasoneurotic constitution as described from Otf. Mül-

ler's laboratory (Parrisius, 1921; Müller, 1922), and adopted by Hagen (1922). It is characterized by the great lability or downright instability of the innervation of the vascular system which manifests itself in the capillaries as well as in the arteries. Frequent changes in the innervation occur either "spontaneously" or from comparatively trivial causes. In the

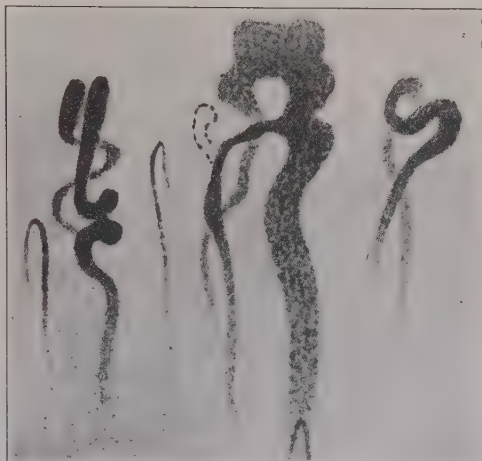


Fig. 72. Capillary loops at the base of the nail. Case of vasoneurosis. After Parrisius.

case histories given by Parrisius it is mentioned how the fingers of certain patients become sometimes quite white (simultaneous contraction of arteries, capillaries, and venules), sometimes strongly red (dilatation of all vessels) or deep blue (contraction of arterioles with dilatation of capillaries and venules).

The innervation varies not only from time to time but also from place to place to a very considerable extent. Capillaries located side by side at the base of the nail, or elsewhere, may show quite astonishing differences, as seen in Fig. 72, where the narrow loops



are nearly normal, while some are enormously dilated. Even within the same capillary vessel there may be dilated portions between others of normal bore, true capillary aneurysms, Fig. 73.

All these symptoms demonstrate the instability of the (sympathetic) innervation and, without committing myself to any opinion, I would point to the sug-

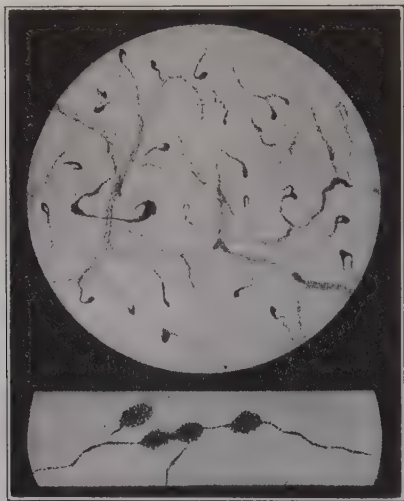


Fig. 73. Capillary aneurysms. After Parrisius.

gestive analogy between these observations and those which have been made on the frog a certain time after section of the nerves to one of the legs or after operative removal of the pituitary gland, and referred to in preceding lectures.

Since the innervation of the skin vessels in the normal subject is by no means constant, there cannot, of course, be a sharp distinction between the normal and the vasoneurotic constitution; and, according to Hagen,

who has examined the capillary circulation in a large number of children and young people, persons who show some deviation from the normal in this direction are by no means rare.

In the severest cases of vasoneurosis, local arterial spasms constitute the most pronounced symptom. These lead to cyanosis, often with very considerable dilatation of the corresponding capillaries and subpapillary venules, and even to Raynaud's gangrene.

Parrisius (1921) and Erben (1918) are of the opinion that a spastic contraction of the subcutaneous veins is largely responsible for the cyanosis in these cases, and Parrisius describes a phenomenon which he believes to be explicable only on the assumption that the subcutaneous veins are closed by contraction. It consists in the fact that when a round spot on the cyanosed skin is pressed with a finger it becomes quite white and the blue color creeps in from all sides like the closing of an iris diaphragm, but not at all from below. This observation is easily explained by the fact that the subcutaneous veins are provided with valves which will not allow the return of blood from them, while there are no valves in the small cutaneous veins. There is no acceptable evidence for a spastic contraction of subcutaneous veins, which must be considered *a priori* rather improbable.

## NOTES

<sup>1</sup> On the palm of the hands little red and white spots are often distinctly visible. These were first described by Ebbecke (1917, p. 51) who noticed the more or less rhythmical change in the color of the single spots taking place one or more times per minute and sometimes reducing a larger area almost to uniformity, sometimes causing a very definite mottling of vividly red and whitish spots.

Lewis (pp. 189-195) emphasizes the fact that the pattern of this mottling is almost constant, not only over short periods on the same day, but from day to day over at least several weeks. We (Rehberg and myself) can confirm this from our own observations, and it is not to be doubted

that generally cutaneous areas in which the tone of capillaries and venules is habitually high alternate with others in which it is habitually lower, and that the extent and distribution of such areas remains remarkably constant.

The constancy of distribution does not, however, mean constancy of tone. Rehberg has studied microscopically the mottling in the hands of several persons. Groups of capillaries comprising 5 to 10 or more loops will suddenly disappear, to reappear after a few seconds, and this process is repeated more or less rhythmically. Adjacent groups sometimes appear simultaneously, sometimes alternately. The changes are probably due to contractions and relaxations of the small vessels themselves, but the possibility that they are of arterial origin cannot be definitely excluded. A fall in pressure caused by arteriolar contraction could not, certainly, empty the capillaries, but would only reduce or stop the flow as often observed in the nail fold capillaries. The dilatation of one arterial branch with the simultaneous contraction of another might, however, bring about plasma skimming into the latter, and, if such plasma skimming is complete, capillary contraction will be simulated.

<sup>2</sup> The adaptation of skin capillaries and presumably of capillaries generally to changes in the effective blood volume appears to come into play only when such changes are relatively large, and one reason for this is no doubt the existence of special and very delicate mechanisms regulating the volume of blood in general circulation, so as not to put any strain upon the veins or capillaries outside the regulating system itself.

By an analysis of a number of different experiments I have shown (1912) that the portal system, comprising the capillaries, venules, and veins of the stomach, intestine, and spleen together with the portal trunk itself, acts as a variable reservoir of blood in virtue of the double set of resistances with which it is provided. When the arterioles supplying this system dilate, blood is transferred to the reservoir, until the portal pressure is raised sufficiently to maintain the corresponding increase in flow through the liver capillaries. Transfusions of large volumes of blood (Worm Müller, 1873; Johansson and Tigerstedt, 1889) are taken up mainly by the portal system, the liver becoming "almost as hard as a board," while the effect outside this system is comparatively slight. Jarisch and Ludwig have recently (1927) studied the changes in volume of an intestinal loop produced by intravenously injecting or withdrawing small quantities of blood. They observe volume changes indicating that the greater part of the quantity added to or abstracted from the general circulation is compensated by the portal system. When, say, 1 cc. of blood is withdrawn the intestinal volume will diminish to such an extent that a large fraction of 1 cc. is restored to the general circulation. This reaction persists after denervation of the intestinal loop experimented upon, but is greatly diminished by denervation of the liver. It follows, therefore, that the second resistance in the system is of paramount importance.

In the liver of the carnivora Mautner and Pick (1915, Mautner, 1924) have demonstrated the existence of a special supply of muscles on the small veins, and their results have been confirmed both anatomically

by Arey and Simonds (1920) and in renewed experiments by Baer and Rössler (1926). These veins contract when exposed to small doses of histamine, and this is shown to be one of the main reasons why histamine causes circulatory shock in the carnivora and fails to do so in rabbits and guinea pigs.

The splendid researches of Barcroft (1926) on the spleen show that this organ has a special function as a regulator of the circulating volume of blood and red corpuscles. During rest it will attain a maximum volume and contain blood with a high percentage of hemoglobin which is renewed very slowly. When circumstances demand an increase in the blood circulation, as, for instance, in muscular work or when muscular work is anticipated, the spleen contracts, as Barcroft has shown, under the influence of the nervous system. To increase the total circulation the pressure in the vena cava must be raised as has been shown (Krogh, 1912) and amply confirmed by the researches on Starling's heart-lung preparation, so that the mechanism investigated by Barcroft must, therefore, to be effective, act in conjunction with the pressor mechanism on the hepatic veins just referred to. When the spleen contracts the hepatic veins must relax and vice versa.

## LECTURE XII

### THE EXCHANGE OF SUBSTANCES THROUGH THE CAPILLARY WALL

**I**N the preceding lectures I have been dealing chiefly with the physiology of the contractile elements in the capillary wall, and I have purposely left out, for the time being, everything pertaining to that which constitutes, after all, the chief function of the capillaries: the exchange of substances between the blood and the tissues, or tissue fluids, taking place through the capillary wall.

Apparently, at least, this function of exchange is a very complex one: gases, water, inorganic salts, organic crystalloids of the most varied description, and, in certain tissues, even colloids are constantly passing through the capillary endothelium, and not infrequently the direction of the passage changes. "After bleeding, the total blood volume in the body is very rapidly recovered. The capillary walls seem to take up the liquid and solid material required, and this material is at the same time reconstituted so as to produce blood plasma of normal composition. . . . If blood is transfused from one animal to another the liquid part of the injected blood is rapidly eliminated." In view of such facts as these, it is, no doubt, tempting to ascribe to the capillaries themselves the power of regulating the passage of substances through their walls, and the statement just quoted from my friend and eminent predecessor as Silliman lecturer, Dr. Haldane (1917, p. 81), seems to imply the belief in such a regu-



lation, which is to him the central object for study in physiological science. I venture to think, however, that the essential aims of physiology are better served by an attempt, however hopeless it may appear, to find causal explanations, to find out by what forces the exchange of substances is brought about, to determine as exactly as possible what the capillary endothelium is capable of doing and what it is not.

What we have to do is, therefore, to see how far the known physical and chemical forces will carry us in attempting to explain the exchange of each single substance through the capillary wall, which we assume to be an inactive permeable membrane. When these forces fail, we have to determine the extent and circumstances of the failure, to discover which substance or substances can be actively transported through the endothelium, in what direction and, if possible, at what rate.

Instead of giving, at this stage, an abstract definition of what I mean by the phrases "known physical and chemical forces" and "active transport," respectively, I think it a better plan to illustrate it by means of examples, chosen from the very domain with which we are dealing.

### *The exchange of gases in the tissues.*

As a case in which the physical forces are certainly sufficient to explain the exchange, I can select nothing better than the supply of oxygen to muscles, the problem which I mentioned in my second lecture by way of introduction to the physiology of capillaries in general.

Oxygen is soluble in water and watery fluids, like blood and tissue fluids generally. In the dissolved state, as well as in the free gaseous state, it will spread, by what is termed diffusion, from any point where its concentration happens to be high to all other points

where the oxygen concentration is lower. The rate of diffusion depends upon the concentration difference. The concentration of dissolved oxygen can be measured by the pressure of the oxygen in an atmosphere with which the dissolved oxygen is in equilibrium, and I define as the diffusion constant for oxygen the number of cc. of the gas which will in one minute diffuse through an area of 1 cm.<sup>2</sup>, when the pressure gradient is one atmosphere of oxygen per  $\mu$  (0.001 mm.). These somewhat arbitrary units have been chosen because they are convenient in physiological applications. Hüfner (1897) measured the rate of diffusion of oxygen through water, and the diffusion constant, calculated from his determinations, is 0.34. I have measured the diffusion through some animal tissues and found the following diffusion constants for oxygen at 20° (1919) :

In Water .....	0.34	(Hüfner)
Gelatine 15 per cent .....	0.28	(Krogh)
Muscle .....	0.14	"
Connective tissue .....	0.115	"
Chitin .....	0.013	"
India rubber .....	0.077	"

These experiments show that the animal tissues are permeable to oxygen, but it is seen that they offer a much greater resistance to the passage of oxygen molecules than does water or even strong gelatine.

The diffusion constant for animal tissues has been found to increase with increasing temperature about 1 per cent per degree between 0° and 40°, taking the rate at 20° as unity.

By means of the determinations of the oxygen diffusion in muscle, combined with measurements given in the second lecture of the distribution and dimensions of muscle capillaries, it becomes possible to calculate the oxygen pressure head necessary to provide a mus-

cle with the amount of oxygen required by it under various conditions.

It was shown in that lecture that in the cross section of a striated muscle we find the open capillaries distributed with conspicuous regularity among the muscle fibers. In a resting muscle only a few are open and the distances between them are considerable in consequence. In a working muscle they are very close together. In either case we can, without committing any serious error, suppose each capillary to supply oxygen independently of all the others to a cylinder

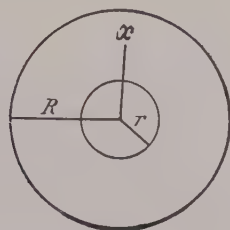


Fig. 74.

of tissue surrounding it. In a transverse section such a cylinder is represented by an area which can be taken as circular, and the average area belonging to each capillary can be calculated by counting the number of capillaries in a transverse section and division of the total area by the number found.

Supposing Fig. 74 to represent the cross section of a capillary ( $r$ ) with the cylinder of tissue ( $R$ ) supplied by it, we shall have oxygen molecules constantly leaving the capillary through the wall and entering the surrounding tissue, where they will be used up at a rate determined by the metabolic processes. The oxygen pressure difference between the inside of the capillary wall and a point at the distance  $x$  from the center of

the capillary must be proportional to the oxygen-metabolism  $p$  and inversely proportional to the diffusion rate  $d$ ; and when these are known, together with the radii  $r$  and  $R$  of the capillary and cylinder, respectively, and the distance  $x$ , it becomes possible to establish a mathematical formula from which the pressure difference can be calculated. Not being much of a mathematician myself, I have asked my friend, the Danish mathematician, Erlang, to work out such a formula for me. It runs

$$T_o - T_x = \frac{10^4 p}{d} \left( \frac{1}{2} R^2 \cdot \log_{\text{nat}} \frac{x}{r} - \frac{x^2 - r^2}{4} \right)$$

in which  $T_o$  and  $T_x$  are the oxygen pressures (in atmospheres) in the capillary and at the point  $x$ , respectively,  $d$  is the diffusion constant as defined above, and  $p$  is the number of cc. of oxygen used up per minute by 1 cc. of muscle; the distances  $r$ ,  $x$ , and  $R$  are measured in cm.<sup>1</sup> Putting  $x = R$  and substituting ordinary logarithms for the natural we get the formula

$$T_o - T_R = \frac{10^4 p}{d} \left( 1.15 R^2 \log \frac{R}{r} - \frac{R^2 - r^2}{4} \right)$$

which will give us the pressure difference necessary and sufficient to supply the whole of the muscle. If this difference is found to be smaller than the oxygen pressure of the venous blood leaving the muscle it follows that every point of the muscle tissue can be supplied with oxygen by diffusion alone: diffusion is *quantitatively* sufficient to cover the oxygen requirements of the muscle.

The pressure heads necessary have been calculated for a small number of typical instances from guinea pig muscles, in which the capillaries and their average distances have been measured. The oxygen consumptions given in the table are more or less arbitrary,

since no determinations could be made in the circumstances. They have been assumed in accordance with the results of determinations by Barcroft and Kato (1915) on dogs' muscles. The two lowest values are to be expected after several hours' complete rest—preferably with the nerves cut. The highest assumed during rest (3 per cent) was observed by Barcroft and Kato on a muscle which had been active under artificial stimulation a short time before the sample was taken. The maximum assumed for work is a little higher than the highest figure actually measured by Barcroft and Kato (13 per cent).

	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>
	O <sub>2</sub> consumption assumed per minute, Vols. % of the tissue	Number of capillaries per mm. <sup>2</sup> cross section	<i>R</i>	<i>2r</i>	T <sub>c</sub> -T <sub>a</sub> in % of atmosphere	Total surface of capillaries in 1 cc. muscle, cm. <sup>2</sup>	Total capacity of capillaries, Vols. % of the tissue
	0.5	31*	100	3.0	6.5	3	0.02
Rest,	1.0	85	60	3.0	3.5	8	0.06
	3	270	33	3.8	2.5	32	0.3
Massage,	5	1,400	15	4.6	0.6	200	2.8
Work,	10	2,500	11	5.0	0.4	390	5.5
Maximum circulation	15	3,000	10	8	0.25	750	15

The oxygen pressure of the venous blood leaving the muscles is probably between 4 and 5 per cent of an atmosphere. When a tension difference of 6.5 per cent is necessary to supply the whole of the muscle there will be oxygen lack in those places which are at the greatest distance from open capillaries. The other cap-

\* This figure has been calculated from an estimation (necessarily somewhat uncertain) of the distance between open capillaries (*2R*) on a living animal. The corresponding value for the diameter of the open capillaries (*2r*) is taken from measurements on a vitally injected preparation with 85 open capillaries per mm.<sup>2</sup>



illary numbers counted during rest give, when combined with the oxygen consumptions assumed, a low positive oxygen pressure everywhere in the muscle.

In a series of experiments undertaken in Barcroft's laboratory, Verzar (1912) has shown that the oxygen consumption of resting muscles depends to a certain extent on the supply of oxygen and decreases when this is diminished. Similar results have been obtained by Gaarder (1918) in my laboratory in determinations of the respiratory exchange of fishes, which have an extremely small number of open capillaries in their muscles. Verzar and Gaarder concluded that the oxygen pressure in parts of the tissues must normally be zero, and it is evident that the countings given in the above table are not at all inconsistent with their result. It is, indeed, probable that the capillary circulation in resting muscles is regulated so as to maintain the pressure at about zero in certain parts which must, however, be constantly changing with the shifting positions of the open capillaries.

After massage and during work the oxygen pressure in the muscular tissue becomes practically equal to that of the blood. This would hold even if higher rates of oxygen consumption were assumed, and it is evident that in these cases the circulation takes place through a much larger number of capillaries than would be necessary to insure the supply of oxygen. It is, therefore, rather probable that the increase in number of open capillaries serves to meet other requirements of the muscle.

In column *f* I have calculated the total surface of the open capillaries in 1 cc. of muscle. This is the area primarily available for diffusion and exchange of substances of any kind whatever between the blood and the tissue. When many capillaries are open this area is seen to be enormously increased.

When diffusion of oxygen through the capillary wall and the tissue cells is more than sufficient to cover the needs of muscles during the heaviest work, it follows that it will probably be sufficient in all tissues, since the available capillary network is, in most active tissues, even closer than in muscles, and it follows a fortiori that the carbon dioxide produced in the tissues can always be eliminated by diffusion into the capillaries, since the diffusion constant for  $\text{CO}_2$  in tissues is some thirty times higher than for oxygen. The  $\text{CO}_2$  pressure difference between any point in the tissue and the blood must, moreover, in all circumstances, be an absolutely negligible quantity.

In a preceding lecture (X) I have discussed the reactions of human skin to occlusion and to moderate increases in temperature and suggested that lack of oxygen might be responsible in both cases for the dilatation of vessels observed.

The arrangement of the cutaneous vessels is in most places too complicated to allow any calculation of the oxygen supply by diffusion, but Spalteholz has given a figure of the papillary and vascular arrangement in the sole of the foot, where the supply is so regular that at least an attempt at calculation can be made. We have in this case papillae of about 0.25 mm. height, and the average distance between the tops of these is about 0.3 mm. Assuming that the capillary loop in each papilla supplies with oxygen a cylinder of germinative tissue in the epidermis the radius of this cylinder will be  $R = 150\mu$ . For the radius of the supplying vessel represented by both branches of the capillary loop I assume a diameter  $2r = 20\mu$ . The diffusion constant of the tissue is taken to be  $d = 0.12$  and the oxygen consumption per minute is obtained from experiments by Gessler (1921, 1922) on the respiratory exchange of excised pieces of pig's skin. Gessler finds on an

average an absorption of 2 mm.<sup>3</sup> oxygen per mg. N in 30 minutes at 37° C. The average nitrogen percentage in the fresh tissue is 3.3 and this works out as 0.22 cc. per 100 cc. per minute. Gessler's values are certainly minimum values, because the pieces of 100-200 mg. used in his respiration experiments were too large to allow oxygen from the atmosphere to reach the interior by diffusion, and it is to be considered further that the germinative layer may probably have a respiratory exchange well above the average. On the other hand some oxygen will certainly reach the epidermal tissue from the subpapillary veins below, and a little will also diffuse in through the horny layers of the epidermis. Using the values given I find  $T_o - T_R =$

$$\frac{10^4 \times 0.0022}{0.12} \left( 1.15 \times 15^2 \times 10^{-6} \log 15 - 10^{-6} \frac{15^2 - 1}{4} \right) = 0.045$$

or 4.5 per cent of an atmosphere. If this figure were accepted, it would mean that at the normal temperature of the skin which is about 30° the oxygen supply would be sufficient to maintain a positive oxygen tension everywhere, but with the increase in oxygen consumption brought about by raising the temperature to 40°, which has been found by Gessler to be about 80 per cent,  $T_o - T_R$  would rise to about 9 per cent which would mean oxygen lack. In view of the uncertainties of several of the elements employed in the calculation it is impossible to make any definite statement, but it does not seem impossible that any large increase in the metabolism of the skin may give rise to oxygen lack and its consequences of vasodilatation.

*The exchange of crystalloids through the capillary wall.*

Although there are a few other substances, such as urea, according to the investigations of Gad-Andresen

(1921), which are probably able to diffuse freely through most of the cells of the body, the gases are the only ones about which we can say with anything approaching certainty that their only means of transport in the body is simple physical diffusion, and for a number of substances we can point out definite instances where the physical forces are clearly insufficient, where molecules are brought steadily and at a considerable rate from a place where their concentration is low to another where the concentration remains considerably higher. As an example, I would mention the problem, raised by Heidenhain in 1891, and much discussed in the following years, of the transport of calcium from the blood to the milk in a cow. The cow produces milk at the rate of 25 l. a day, with a calcium content of 42.5 g. or 1.7 g. per l. This quantity of calcium is derived from the blood, in which the calcium content does not exceed 0.18 g. per l. The calcium ions cannot possibly move spontaneously from the blood to the alveoli of the mammary gland. Their transport must involve the expenditure of energy, must involve work performed in living cells by means of some special "machinery," so far utterly unknown, adapted for that purpose; must involve, in short, *secretion of calcium*.

The point I wish to emphasize in connection with this and the many other well-known examples of glandular secretion is that there is no need to assume, indeed, there is no cogent reason to suppose, that any part of the secretory work is done by the capillary endothelium. We have the gland cells for that, large, robust cells with a complicated protoplasmic structure, a structure which we can, however dimly, recognize as having something to do with the secretory work the cells are called upon to perform; and all that is required of the capillary endothelium is that it shall be

permeable to the substances which the gland cells take up and secrete. When, in the above example from the mammary gland, the gland cells absorb calcium ions so quickly that their concentration outside the endothelium is kept definitely lower than in the blood, there can be no doubt that, with the enormous endothelial surface available, the endothelium need be only moderately permeable to calcium ions to provide all the calcium required by the gland.

Still another point comes out clearly from this discussion: The glands are not well suited for a study of the properties of capillary endothelium, since we cannot *experimentally* separate the endothelial functions from those of the gland cells. For the purposes of such a study, tissues must be selected where we can bring a fluid in direct contact with the outside of capillaries and investigate the exchange of substances between such a fluid and the blood. The subcutaneous tissue is one of the places where such an interchange of substances can be conveniently studied. It has been made use of in a very large number of experiments and is being used many thousand times every day by physicians for the introduction of the most various substances into the blood of patients. The general result of all the experiments, consciously and unconsciously made, is that all crystalloid substances pass freely through the capillary endothelium in both directions.

Usually the injected substances appear in the blood after such a short interval of time that absorption by way of the lymph vessels can be excluded. In a small number of cases it has been ascertained by special precautions that the substances in question were, in fact, taken up directly through the capillary wall. In many experiments a passage of substances has been recorded *from* the blood to an artificially injected fluid or a natural edema. In those cases where the point was



directly studied concentration equilibrium was obtained, or at least approached, for the substances involved, between the blood and the outside fluid.

Results similar to those obtained by means of subcutaneous fluids have been obtained from injections into the abdominal cavity or pathological cases of ascites, in spite of the fact that in this case the diffusing substances must traverse the peritoneal epithelium in addition to the capillary endothelium.

In a number of experiments, made in the nineties by Heidenhain and his school and by Cohnstein, diffusible substances were injected into the blood and comparisons were made between their concentrations in the blood and in the lymph from the thoracic duct at different periods after the injection. In many of these experiments the concentrations were found to be somewhat higher in the lymph than in the simultaneous sample of blood, and in some it was even higher in one of the lymph samples than in any of the blood samples. This was assumed by Heidenhain and his school to be due to active secretion from the capillary blood into the lymph, though it was admitted that diffusion took place also. I believe it is generally agreed now that such a conclusion cannot be final, in view of the unsurmountable difficulties in the way of determining the real correspondence in point of time between the samples of blood and lymph, respectively, and the further difficulty of hitting the real concentration maximum in each fluid by means of a comparatively small number of samples taken at ten- or twenty-minute intervals.

Authors who believe in the secretory powers of the capillary endothelium have mentioned several cases in which they consider that active transport of substances must have taken place (e.g., Volhard, 1917). It would serve no useful purpose to consider these in detail, because the conditions are generally, as in the

above example, too obscure to allow any valid conclusion being reached, and when I review all the facts that have come to my notice I have no hesitation in saying that there is no trustworthy evidence of the capillaries having any power of hindering or favoring the passage by diffusion of all kinds of crystalloids through the endothelium.

The rates at which different substances will diffuse through the capillary wall seem to be closely related to their rates of free diffusion in water or gelatine. For some inorganic salts and for glucose Clark (1921) has arrived at this conclusion by an interesting series of experiments, to which I shall refer in some detail at a later stage of my argument (Lecture XIII).

In a very important contribution to our subject Schulemann (1917) has studied the rates at which organic dyes, subcutaneously injected into mice or rabbits, are distributed over the whole organism and taken up by certain cells. He compares these rates with the rates at which the same dyes will diffuse through gelatine and finds a general and close correspondence. Since the dyes are distributed by the blood, after passing into the capillaries at the place of injection, and stain the cells by which they are taken up, after renewed passage through the endothelium, Schulemann's results show that the passage takes place by diffusion and that the relative rates of diffusibility are the same through the capillary endothelium as through gelatine.

The general significance of the permeability of the capillary endothelium to crystalloids is obvious: it secures the supply of all the substances, which may be required for the intracellular metabolic processes, at the very surface of each cell. If any substance is taken up by a cell for storage, conversion, or transport, its concentration in the tissue fluid at the surface of that

cell is lowered, and the lowering leads automatically to a diffusion of that particular substance toward the surface where it is required.

*The impermeability of the capillary wall to colloids.*

Schulemann has found that dyes that are unable to diffuse from a watery solution into gelatine will not, when injected subcutaneously into an animal, produce any general vital staining, but remain at the place of injection, where they may be taken up by certain cells. These dyes are clearly unable to diffuse through the capillary endothelium, and the same appears to be the case with regard to colloids generally: they are unable to diffuse through the normal capillary endothelium in most organs. Certain exceptions to this rule will be dealt with in the next lecture.

From our physiological point of view the greatest importance attaches to the impermeability of capillaries to the normal colloids of the blood—the proteins. When a protein solution is injected subcutaneously the absorption is extremely slow and apparently does not take place at all through the capillary endothelium.

I. H. Lewis (1921) has made subcutaneous injections of serum which he was able to recognize by a “complement binding” reaction, sensitive to one part in a million. He found the specific protein in the thoracic duct after forty minutes, but it could be detected in the blood only after three and a half hours.

Protein-free fluids, injected subcutaneously, remain protein free until they are absorbed, and pathological edema fluids *may* remain practically protein free for an indefinite period, as shown, for instance, by a case of ascites mentioned by Volhard (p. 1478).

*The exchange of water through the capillary wall.*

The impermeability of the capillary wall for colloids forms the basis of the mechanism for absorbing iso-

tonic solutions of crystalloids into the circulation, described in 1896 in the classical paper by Starling: "On the absorption of fluids from the connective tissue spaces."

To demonstrate the absorption of a salt solution, isotonic with the blood, from the tissue spaces directly into the capillaries, each of the surviving hind limbs of a dog was perfused with the dog's own defibrinated blood, which was made to circulate regularly through the leg. One of the legs was first made edematous by the injection of 1 per cent NaCl solution, and it was found that, while the blood circulating through the normal leg remained practically unaltered, the blood circulating through the edematous leg became gradually more dilute by taking up the fluid from the edema.

This absorption seemed very puzzling, since the initially higher osmotic pressure of the outside fluid must cause water to pass from the blood into the tissue spaces, while the constantly higher hydrostatic pressure of the blood in the capillaries must set up a filtration of water and salts in the same direction. Starling showed that the explanation of the observed absorption lay in the osmotic pressure of the blood colloids.

It is unnecessary here to go into the question about the exact nature of osmotic pressure, which is still a debated problem, and I need only remind you that the term osmotic pressure expresses the attraction of dissolved substances for the solvent fluid; that its existence can be demonstrated when the (watery) solution is separated from pure water by a membrane, permeable to water, but not to the dissolved substance, in which case the pure water will pass continuously through the membrane into the solution, which increases in volume and becomes more dilute. When the solution is put under pressure a filtration of water will

take place in the opposite direction and, when the pressure is increased to such a height that the filtration just balances the osmotic current of water and the volume of the solution neither increases nor decreases, the filtration pressure set up is equal to and can be used as a measure of the osmotic pressure of the solution.

The osmotic pressure of a solution of any pure substance depends upon the *number* of molecules, ions, or other particles present, quite irrespective of their kind or size, and for one gram molecule of an undissociated substance (180 g. glucose, for instance) dissolved in one liter of water it is equal to 22.4 atmospheres.

The osmotic pressure of a solution containing a mixture of substances is made up of the partial pressures of the single substances each of which acts as if it were alone present, provided, of course, that the total number of free particles is not modified by reactions between the substances.

The osmotic pressure of human blood amounts to about 6.5 atmospheres. Of this pressure by far the greater part is due to the inorganic salts dissolved in the plasma, and most of the rest to organic crystalloids. The blood sugar, for instance, amounting to about 1 g. per l., exercises an osmotic pressure of 0.125 atmosphere or 1.3 m. water pressure. Starling showed, however, that even the proteins, which make up practically the whole of the colloids of the blood, have a definite though small osmotic pressure.

The osmotic pressure of the crystalloids cannot become *effective* in normal capillaries, because their walls are permeable to these substances which will pass through by diffusion until an equilibrium is established. The osmotic pressure of the proteins on the other hand is effective by virtue of the impermeability of the capillary walls for colloid substances.



The osmotic pressure of the blood colloids—the proteins—can be exerted and measured when the blood is separated from a protein-free solution, containing the blood salts, by a membrane which is impermeable to proteins.

Starling constructed osmometers from small glass bells provided near the top with two vertical tubulures. Over the mouth of the bell was tied a peritoneal membrane, which was soaked in 10 per cent gelatine for some minutes after it had been tied on. The membrane was prevented from bulging by fixing it over a perforated silver plate. One of the tubulures was connected either with a long, narrow, vertical tube or with a small mercurial manometer. These osmometers were filled with serum and their lower ends allowed to dip into a salt solution, which was generally chosen so as to be slightly hypertonic. In these circumstances the fluid in the vertical tube would sink a little at first, but in all the experiments it began to rise within two or three hours and rose steadily for three or four days, the final height varying from 30 to 40 mm. of mercury or 400 to 550 mm. of water. When the osmometers are started with a pressure higher than this, filtration of salt solution takes place until the same point of equilibrium is reached.

In the capillary blood vessels we have, just as in the osmometer, a membrane which is permeable to crystalloids and impermeable to colloids. An absorption of isotonic salt solution can, therefore, take place, and, indeed, must take place, when the hydrostatic pressure in the vessels—the capillary blood pressure—is lower than the osmotic pressure of the proteins. Since the surface of the capillaries available for the osmosis is, as we have seen, very large, a rapid absorption of salt solution can be effected by this mechanism, but to study the exchange of water more closely we must obtain

more detailed information about the osmotic pressure of the colloids of the blood and about the filtration pressure of the blood in different capillaries.

Since Starling's publication the osmometers for colloids have been repeatedly improved and more accurate determinations of the osmotic pressure of the blood colloids made, but nothing of a very essential nature has been added to Starling's explanation.

*The colloid osmotic pressure of the blood.*

Theoretically there is no sharp line of distinction between crystalloids and colloids; every gradation exists between the size of the smallest ions and microscopically visible particles. In the blood, however, there is, as I shall show presently, a very definite gap between the smallest protein molecules for which the capillary wall is normally impermeable and the largest crystalloids which penetrate without difficulty. As osmotically active substances we have to deal only with the proteins, and it will be appropriate to discuss briefly the osmotic pressures of pure proteins, as studied chiefly by Sørensen (1917) and Loeb (1922).<sup>2</sup>

The experiments and theoretical considerations of these authors show that the osmotic pressure of proteins is governed mainly by the Donnan effect depending upon the existence of protein salts with acids and alkalis and the ionized state of these salts. At a certain concentration of hydrogen ions, the isoelectric point (or zone), characteristic for each protein (for crystalline egg albumin at  $p_H = 4.8$ ), the protein molecule is either uncombined or its salts un-ionized. In less acid solution (higher  $p_H$ ) the protein behaves as an acid in combination with alkali and with more alkali the higher the  $p_H$  up to a certain point. The osmotic pressure of such a solution will depend not only upon the number of protein particles or molecules, but also

upon the metal ions which, though otherwise able to penetrate the membrane, are held back by the electric attraction between them and the protein ions. The osmotic pressure of a protein solution is, therefore, normally higher and can be much higher than the pressure directly corresponding to the number of protein particles.

It follows further from the Donnan theory and has been confirmed experimentally that the osmotic pressure of a protein solution is not only determined by the  $p_H$  but is also influenced by the presence of neutral salts and that, other things being equal, it cannot be expected to be simply proportional to the protein concentration, but will show more complicated relationships between pressure and concentration. In the absence of neutral salts and when their concentration is very low the total pressure per gm. per cent increases considerably with the concentration as found by Loeb.

Different proteins differ considerably as to their osmotic pressure, and when a number of precautions are observed osmotic pressure measurements can be utilized to measure the "molecular" weight of proteins in solution. Adair (1926) has deduced a molecular weight of 62,000 for serum albumin, 130,000 to 150,000 for pseudoglobulin, and 174,000 for euglobulin. The globulins isolated are scarcely pure substances, but it is important from our point of view that they have certainly larger molecules and exert weight for weight a smaller osmotic pressure than the albumins (Farkas, 1926).

In the blood a variable mixture of albumins and globulins is responsible for the colloid osmotic pressure, which is moreover complicated by the presence of salts and definite though slight variations in the hydrogen ion concentration. It is not, at present, possible to deduce from the determinations on pure proteins the

variations to be met with in the blood or to calculate with sufficient accuracy the osmotic effect from determinations of total proteins and the albumin-globulin ratio as has been attempted, Govaerts, 1927. We have in my laboratory (Krogh and Nakazawa, 1927) made a number of determinations to test empirically the relation between the colloid osmotic pressure exerted by serum and the factors which are known to be variable. We found that the temperature has an extremely slight influence, the colloid osmotic pressure of mammalian serum being scarcely measurably lower at room temperature than at body temperature. An influence of hydrogen ions, neutral salts, and carbonic acid could not be detected within physiological or even pathological limits of variation ( $p_H$  8 — 6.5, 0.3 — 1.5 per cent salt, 0 — 20 per cent  $CO_2$  pressure.<sup>3</sup> These results confirm and extend the corresponding experiments of Mayrs (1926). They are of considerable importance technically, because they allow determinations to be made at ordinary temperatures and without much attention to conditions regarding loss of  $CO_2$  or other changes in  $p_H$ .

In spite of the fact that neutral salts are present in sufficient quantity to minimize the increase in the Donnan effect, otherwise to be expected with increasing concentration of protein, the colloid osmotic pressure of blood or serum calculated per gm. protein per cent increases with the concentration. This increase was studied by Mayrs (1926) and Verney (1926) and our results confirm and extend their observations as summarized by Fig. 75 in which curves are drawn to represent the pressure per gm. per cent at varying concentrations of the same serum, albuminous urine, or other colloid solution.

A satisfactory theoretical explanation of this relation between concentration and osmotic pressure cannot be given at present (Cf. Verney, Krogh and Na-

kazawa, Marrack and Hewitt, 1927) but it must clearly be taken into account, when sera with different protein contents are compared as in the study of pathological cases.

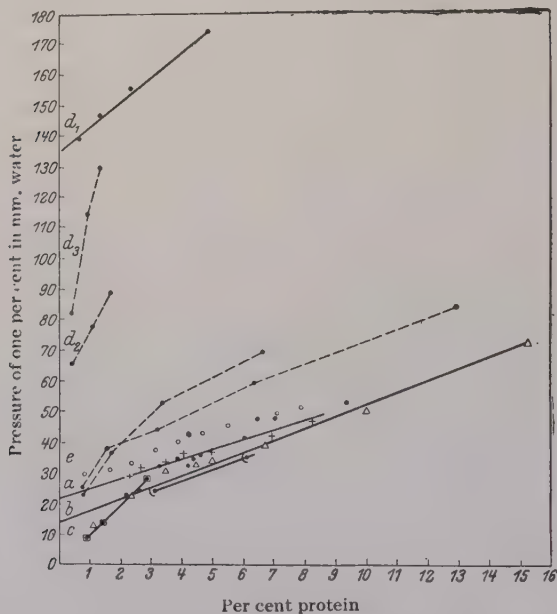


Fig. 75. Colloid osmotic pressure of different fluids calculated for 1 per cent colloid at the concentrations indicated. *a* Human serum. + Determinations by Krogh and Nakazawa. *o* Determinations by Mayrs. . Determinations by Verney. *b* Standard horse serum. *c* Frog's serum. *d* 1-3 Human urines. *e* Eggwhite. ( . Gum acacia solution. Krogh and Nakazawa.

We have in my laboratory done a very large amount of work, of which a preliminary account was given in the first edition of this book, to determine what we called the fractional osmotic pressure of the blood colloids, but we have had to abandon the attempt, because we found the technical difficulties unsurmount-



able, although the principle was shown to be sound and to be applicable in certain cases to processes in the organism, as we shall see in a later lecture. The conception was that the osmotic pressure could by the use of collodion membranes of graded permeability be split up into fractions each due to particles above a certain size, defined by the pores of the membrane in question. We succeeded in showing that there is a very definite colloid fraction, since we found a certain pressure by using membranes which are just impermeable to proteins, exactly the same pressure in membranes with much smaller pores, and a measurably lower pressure as soon as the outside fluid gives any protein reaction.<sup>4</sup>

We can show further that there is a definite colloid fraction with a negligible osmotic pressure—the fibrinogen fraction, since we find that oxalated or hirudinized plasma of an animal exerts the same pressure as the defibrinated serum. We find, for instance, on the plasma of a rabbit with 6.5 per cent protein a pressure of 332 mm. water and on the serum from the same blood with 6.2 per cent protein a pressure of 334 mm.

The separation of definite colloid fractions by means of graded membranes failed, because we could not make or obtain membranes of sufficient uniformity. It is easy to get a membrane that will let through certain smaller protein molecules and give in repeated determinations the same fractional pressure, but we have found it impossible to reproduce such membranes. A series of membranes made exactly alike and with the same filtering capacity for water (as defined in the appendix, p. 384) of, say, 6 cc. will give results for the osmotic pressure which are all lower than that of the 1 cc. membrane, but too divergent to be of any use. We can only conclude, therefore, that colloid molecules and aggregates of all possible sizes are present in the blood. We have reason to believe that generally the

serum albumin as it exists in the blood is made up of smaller particles than the globulins and is responsible for the larger fraction of the osmotic pressure, but attempts to calculate the pressure from determinations of the albumin and globulin fractions are bound to fail because there is within each a large assortment of different-sized particles.

The colloid osmotic pressure differs considerably in different animals. I have collected a number of determinations partly from the literature and partly from the experiments done in my laboratory in our attempts to fractionate the blood colloids.

In the brown frog (*Rana temporaria*) the protein content of the blood and its colloid osmotic pressure are both very variable according to the season and the condition of the individual frog and the variations are not always parallel. The normal values for the serum seem to lie about a protein content of 2.5 per cent with an osmotic pressure of 70 mm. water. In an emaciated specimen Churchill, Nakazawa, and Drinker (1927) measured an osmotic pressure of only 15 mm., corresponding to a protein percentage of 1.4, but we have repeatedly found that blood with such a low protein content may show a normal pressure. We have seen two cases of blood with a protein content of 4.1 to 4.3 per cent with pressures of 140 and 135 mm. On the bullfrog (*R. catesbiana*) White (1924) has made three determinations which I reproduce, because they have been used and confirmed in the work of Landis presently to be discussed. He found protein percentages of 2.40, 2.52, and 2.80 with corresponding pressures of 96, 98, and 115 mm. of frogs' plasma.

In mammalian serum the pressures are higher and much less variable. In seven rabbits we found an average protein content a little below 6 per cent with a pressure of 270 mm., the lowest being 4.8 per cent with

230 mm. and the highest 6.2 per cent with 330 mm. In five cats we found on an average 7.4 per cent and 310 mm. with variations from 6.6 to 8.4 per cent corresponding to pressures of 260 and 300 mm., respectively. The highest pressure measured was 345 mm. in a serum with only 6.8 per cent protein. In the ox our values on five animals range only from 6.8 to 8.2 per cent protein with pressures from 340 to 380 mm.

On man numerous determinations have been made by different observers and there is a wide divergence in individual results as is to be expected when the function studied is variable. The values range from below 300 to over 500 mm. water pressure. They are on the whole unmistakably dependent upon the protein concentration, but in individual cases the relation is often obscured by the variations of the osmotic qualities of the protein.

The average results of different investigators show considerably larger differences than those that can be accounted for by the individual variability, and these must be due to the technique and in certain cases to the permeability of the membranes used. The highest values have been found by Dieter (1925) working on hirudinized blood and employing the elaborate and accurate technique of Sørensen. His average from 54 normal cases is 420 mm. with variations from 370 to 480 mm. Unfortunately he has given no determinations of the protein content. Mayrs (1926) found on the mixed hirudinized plasma of several individuals a pressure of 410 mm. corresponding to a protein content of 7.9 per cent. In my laboratory we have in determinations on serum obtained in 12 cases an average pressure of 380 mm. with a protein content of 7.65. These figures include the normal cases of Iversen and Nakazawa (1927, eight cases, 360 mm., 7.56 per cent). Govaertz (1924) finds an average of 380 mm. in

10 cases with an average protein content of 8.15, while Serr (1924) reports a pressure of 370 mm. as the average of 72 cases. His protein values, which must be too high, range from 8.2 to 10.3 per cent and the osmotic pressure found is too low by an unknown amount, because practically all his membranes were slightly permeable to protein. Runge and Kessler (1925) report 367 mm. (27 mm. Hg) as the average for their normal cases, studied with the technique of Schade. The lowest values reported are those of Schade and Clausen (1924). The determinations on the serum of 10 cases range from 280 to 370 mm. with an average of 340. The technique employed, though otherwise beautiful, excluded the possibility of testing the outside fluid for protein.

I have no doubt that the lowest values of this series are in error, and I am inclined to favor the highest as probably correct, but the subject evidently requires further study. For many purposes the relative values obtained with a constant technique are quite sufficient, but in other cases absolute values are essential as we shall see presently.

## NOTES

<sup>1</sup> Professor Parsons has given me the following account of the steps used in arriving at this formula.

When even the outermost layer of tissue included in the cylinder is just adequately supplied with oxygen the pressure gradient of the diffusing gas will be of such magnitude that the rate at which diffusion takes place across any cylindrical surface within the tissue cylinder (say that with radius  $x$ ) must be equal to the rate at which oxygen is used between this surface and the outer surface (radius  $R$ ). The equation, thus expressed in words, can be put into mathematical symbols.

Considering a length  $l$  of the capillary and its surrounding tissue cylinder we find the rate at which oxygen is used by the tissue included between the surface ( $x$ ) and the outer boundary ( $R$ ).

$$(\pi R^2 l - \pi x^2 l) p \text{ cc. per minute}$$

while the rate at which oxygen is diffusing through the cylindrical surface  $x$  is

$$-\frac{dt}{dx} \cdot 2\pi x l D$$

where  $2\pi x l$  is the total area of the surface  $x$ ,  $D$  is the diffusion constant for oxygen in the tissue expressed in cm. units, and  $-\frac{dt}{dx}$  is the unknown

rate of change of oxygen tension with distance at the distance  $x$ , i.e., the oxygen pressure gradient at the distance  $x$ —negative because as the distance increases the oxygen tension diminished.

From what we have just explained these two quantities are equal

$$-\frac{dt}{dx} 2\pi x l D = (\pi R^2 l - \pi x^2 l) p.$$

This differential equation is solved by separating the variables  $T$  and  $x$  to opposite sides. At the same time eliminating  $\pi$  and  $l$  we have

$$-dt = \frac{p}{2D} \left( R^2 \frac{dx}{x} - x dx \right).$$

Integrating between the limits  $x = r$  where  $T = T_0$   
and  $x = x$  where  $T = T_x$

we have

$$-\int_{T_0}^{T_x} dt = \frac{p}{2D} \left[ R^2 \int_r^x \frac{dx}{x} - \int_r^x x dx \right]$$

$$\text{or } T_0 - T_x = \frac{p}{2D} \left( R^2 \log_{\text{nat}} \frac{x}{r} - \frac{x^2 - r^2}{2} \right).$$

As stated above  $D$  is the diffusion constant of oxygen expressed in terms of a pressure gradient of 1 atmosphere per cm. Substituting for this our diffusion constant expressed in terms of atm/ $\mu$  we have  $D = \frac{d}{10^4}$  and we find

$$T_0 - T_x = \frac{10^4 p}{d} \left( \frac{1}{2} R^2 \log_{\text{nat}} \frac{x}{r} - \frac{x^2 - r^2}{4} \right)$$

<sup>2</sup> Certain authors (Ellinger and Heymann, 1921, Schade and his collaborators) make a sharp distinction between the osmotic pressure of crystalloids which are truly dissolved and the "imbibition pressure of colloid sols" which they hold to be essentially the same as the pressure exerted by the swelling of gels, and Schade (Schade and Menschel, 1923) has coined the word "oncotic" pressure to emphasize the distinction. Ellinger and Heymann attempted to show by perfusion experiments on



frogs that the "imbibition pressure" of small quantities of serum proteins added to their perfusion fluids was much higher than what could be exerted osmotically by the same substance in vitro. Their results have been explained, later, through the experiments of Drinker to be described in the next lecture. Schade measures the "oncotic" pressure in the legitimate way in collodion osmometers and we have apparently to do only with a difference in nomenclature. Nomenclature should not be used, however, to complicate relations which are in themselves difficult enough. The experiments of chemists like Sørensen, Loeb, and Svedberg leave no room for doubt concerning the essential identity of the processes resulting in osmotic pressure of colloids as well as of crystalloids.

<sup>3</sup> Schade (1927) emphasizes the fact that in whole blood the changes in  $p_H$  taking place during the passage through the systemic capillaries will cause the corpuscles to take up water from the plasma. The consequent increase in concentration must bring about a corresponding increase in its colloid osmotic pressure which is thought to be very important from the point of view of exchange of fluid with the surrounding tissue. It is difficult to see how even a distinct increase should be able to exert any essential influence and in any case the effect is so slight (less than 1 per cent according to L. J. Henderson, Bock, Field, and Stoddard, 1924) that it is entirely negligible from the point of view of the osmotic pressure.

<sup>4</sup> In our initial experiments we found a definite increase in pressure when substituting membranes with a filtering capacity for water of about 0.1 cc. per minute per 100 cm.<sup>2</sup> at 1 atmosphere for those of 1 cc. filtering capacity which are normally impermeable to protein, but this turned out to be a mistake, due to sources of error which have since been detected and eliminated (Krogh and Nakazawa).

## LECTURE XIII

### THE EXCHANGE OF WATER BETWEEN THE BLOOD AND THE TISSUE SPACES

#### *The capillary blood pressure.*

**T**HE hydrostatic pressure of the blood in the capillaries determines, by its relation to the osmotic pressure of those colloids for which the capillary wall is impermeable, the direction and rate of exchange of water (isotonic salt solution) between the tissue spaces and the blood. When the capillary blood pressure is higher than the osmotic pressure an excess of water over that attracted osmotically will filter out through the capillary walls, and edema will develop. When the capillary pressure is lower than the osmotic pressure any excess of fluid in the tissue spaces will become absorbed. The rate at which such an absorption can take place depends upon the difference between the osmotic pressure and the capillary blood pressure.

In this preliminary statement I have taken no account of two factors in the exchange of fluid, viz., the tissue pressure outside the blood vessels and the osmotic effect of colloids present in the tissue spaces. As we shall see later on these factors are in most cases negligible, though there are instances where they influence the course of events to a marked degree.

According to the original conception of Starling which has been recently revived and elaborated by Schade (1927) the pressure at the arterial end of a normal capillary system should exceed the colloid os-

motie pressure with a resultant filtration of water; at some point within the capillaries equilibrium should be attained, and at the venous end the conditions should be reversed and water absorbed. This would mean a semi-edematous state near the arterioles and a constant extracapillary flow of water from the arterial end of any capillary toward the venous side. Schade seems to consider such a flow as essential for the capillary function of exchange, but this view is certainly erroneous. The exchange takes place by constant movement of ions and molecules including the water molecules in both directions through any point of the capillary wall. We have to do only with the resultant of these movements and the special case of the water molecules. According to the conception of Schade the mean capillary pressure should not be very different from the colloid osmotic pressure. Schade (1927) assumes a range of pressures within the single capillary from about 60 mm. Hg at the arterial end to less than 10 mm. at the venous end, and it would follow that any increase in the capillary pressure or any diminution of the attraction for water of the blood colloids must cause an excess filtration outward through the capillary wall of a volume of water which could only be removed through the lymphatic vessels.

The numerous microscopic observations of living tissue made in my laboratory have led me to a different conception, briefly alluded to in the first of these lectures. We find that the vascular bed in the arterioles is normally extremely narrow, compared both with that of the larger arteries and with the capillaries and veins. We cannot doubt, therefore, that the main resistance to be overcome lies in the arterioles where the main fall in pressure must accordingly take place, while comparatively insignificant pressure differences must suffice for the current through capillaries and

veins. These observations have been made on the skin of man, rabbit, and frog, on muscles of several animals and on the bladder and intestine of the frog, while the mucous membrane of the frog's tongue and the frog's mesentery form notable exceptions in which the relative constriction of the vascular bed in the arterioles is much less pronounced.

I was led to assume from such observations that the pressure in most capillary systems would be everywhere lower than the colloid osmotic pressure of the blood. This assumption was supported by the known facts concerning absorption from tissue spaces, but to decide the issue direct pressure determinations were necessary.

The determinations of capillary blood pressure found in the older literature are very discordant and uncertain. Most of them have been made on the human skin by observation of the pressure necessary to obstruct the flow or blanch the skin, and it has been shown (Landis, 1926; Klingmüller, 1927; Lewis, 1927, 2) that by these methods results may be obtained which can be both a great deal higher and notably lower than the actual pressures which it is desired to measure. Even the method of Basler (1914), who makes an incision into the vessel in which the pressure is to be measured and notes the pressure which is just sufficient to stop bleeding, will often give too high results, because the capsule transmitting the outside pressure may cause obstruction to the venous outflow and artificially raise the blood pressure.

The first direct and reliable measurements of capillary blood pressure were obtained on the human skin by Miss Carrier, working with Rehberg in my laboratory (1922). She pierced the skin and the top of a favorably situated capillary loop with a glass capillary tube containing saline under an adjustable pressure.

Holding the tip of the glass tube within the blood current for some seconds she observed whether blood would run into the glass tube or not. When the blood enters the tube its pressure is evidently the higher and in such a case the pressure is raised by several cm. water and another capillary loop tried. If the blood here fails to run into the glass needle, two or three more loops are tried to confirm this result and the pressure is then lowered to an intermediate point. By narrowing down the limits between which the blood does or does not run up against the pressure, it is possible to come within  $\frac{1}{2}$  cm. of the pressure in the capillaries. Occasionally the pressure in the capillary is just equal to the pressure in the glass needle. When such is the case the blood pulsates in the tip of the glass needle with every beat of the heart.

This method has been controlled in several ways and found to be absolutely reliable. Its validity has been called in doubt (Kylin, 1926) on the ground that the glass tube will obstruct the flow in the capillary in question. This is certainly so in some cases, and if we assume that the tube obstructs completely the inflow from the arterial loop we have to look upon the venous loop and the glass tube as a closed side tube to the nearest venule. The pressure in such a case is, therefore, that of the first net of venules instead of that of the top of the capillary loop. This difference is too small to be detected.

The capillary and venule pressure is found to be greatly influenced by the vertical position of the point on the surface where the determination is made relative to the thoracic cavity. A series of determinations of capillary pressure on the hand of one of the subjects (*A.R.*) will make this clear. The position of the hand relative to the center of the clavicle is given in the table. Positions above the clavicle are indicated by —,



below by +. The pressures are given both in cm. water and (by division with 1.05) in cm. blood.

Position,	-20	+1	+7	+8	+12	+19	+33.5	+36
Pressure { blood,	4.3	4.3	4.3	5.7	9.5	16.2	27.5	30.5
{ water,	4.5	4.5	4.5	6	10	17	29	32

The pressure is seen to be constant and very low, from 7 cm. below the clavicle, upward. Below that point it increases regularly with the increasing vertical distance. The results are shown graphically on the chart, Fig. 76.

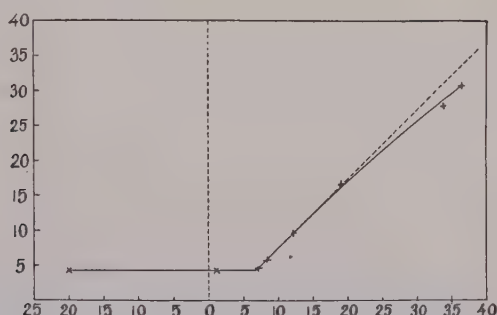


Fig. 76. Capillary pressure in hand at different levels above and below clavicle. Dotted line: Theoretical hydrostatic pressure. After Carrier and Rehberg.

In another subject (*P.R.*) the neutral point was about 10 cm. below the clavicle, and the pressure measured at or above this level was 7.5 cm. water. A few other subjects showed minimum capillary pressures in the hand between the limits 4.5 and 7.5 cm. water.

To understand why the capillary pressure becomes constant above a certain level one must bear in mind the slightly negative pressure in the thorax and the peculiar collapsible structure of the veins connecting

the capillaries with this cavity. The pressure is made up of two components: the hydrostatic pressure, due to the difference in level between the capillary system and the thoracic cavity, and the frictional resistance pressure, determined by the total cross-section of the veins and the velocity of flow. When the hands are lifted above a certain level the veins begin to collapse, with the result that the frictional resistance is increased. As the walls of the veins are quite soft and yield to the slightest excess of pressure from outside, the negative hydrostatic pressure which would be set up in a rigid tube when lifted above the level at which it enters the chest is automatically compensated by the increase in frictional resistance in the venous system, which collapses more and more as the hand is raised. It is a simple physical consequence, therefore, of the conditions of flow in collapsible tubes that the capillary pressure must become constant at all levels above that at which the veins begin to collapse.

Postponing for the time being the consideration of the high pressure brought about hydrostatically by the weight of a column of blood in the veins between a capillary system at a low level and the heart we find the impression gained by simple microscopical observation fully confirmed by the pressure determinations on normal skin.

When the blood flow is increased by dilatation of the arterioles an increase in the capillary pressure must necessarily result, and I have made determinations by three different methods of the venule pressure in "arteriolar" reflex flare produced by pricking in histamine. A single determination was made by the Carrier method and showed a pressure below 15 cm. of water. A small number of determinations were made by means of the pulse pressure method described in the appendix (p. 390) and showed pressures varying be-

tween 10 and 18 cm. at a point 10 cm. below the clavicle, and determinations by a modified Basler method gave similar minimum values while others were higher. In some of these latter experiments it was evident that arterioles had been cut open.

The rise in pressure is, therefore, perceptible and relatively quite large, viz., from the normal of 7 cm. to an average of about 12 cm. It should be remembered that in this case the capillaries and venules are themselves actively relaxed (cf., p. 131). In cases of a purely arteriolar dilatation, where the color of the skin remains unaltered, the pressure in the capillaries will undoubtedly rise to a higher figure, but it seems probable that even then the pressures along the whole length of the cutaneous venules will fall short of the colloid osmotic pressure of the blood. Otherwise filtration edema would develop. The point of equilibrium between the colloid osmotic pressure and the blood pressure is not normally found in the capillaries, but somewhere in the arterioles where the surface is small. The whole of the system of venules is available for absorption and there is a broad margin of safety to cover an eventual dilution of the blood or an increase in venous pressure.

A most beautiful series of determinations has been made by Landis (1926) on the mesentery of the frog where, as I have said, conditions differ from those in the human skin, in so far as the total vascular bed is much less expanded on the transition from arterioles to capillaries. A further difference is due to the fact that the mesenteric veins belong to a portal system with a secondary resistance in the liver. Landis has improved the micro-injection technique of Miss Carrier and, utilizing the micro-manipulator of Chambers, he is able to make determinations in any vessel with the utmost precision and apparently with great ease.

Conditions of flow in the mesentery are very variable, but from a large number of determinations very fair averages can be obtained. Landis summarizes his average results in Fig. 77 which will repay a close study. On 6 mm. of artery the pressure remains practically constant over the whole length, though with a wide

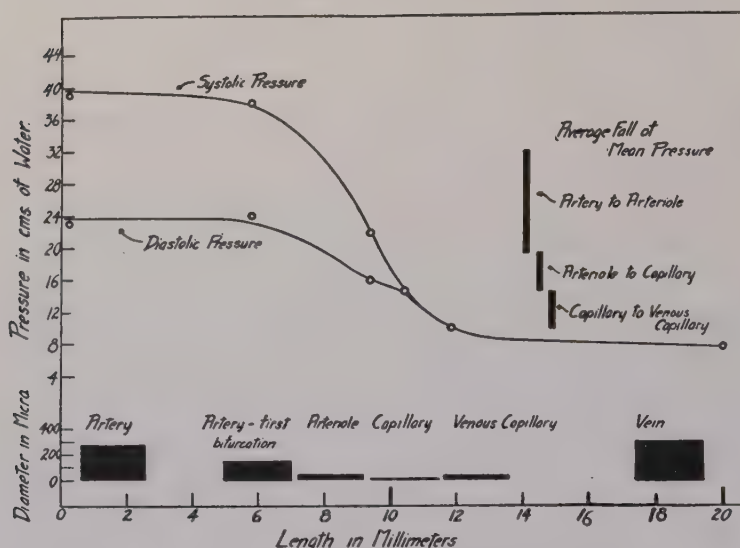


Fig. 77. Average fall in pressure through the vessels of the frog's mesentery. After Landis.

divergence between systolic and diastolic pressure. The main fall takes place in the arterioles, but it is continued through the whole of the capillary system, occupying a length of not less than 3-4 mm. which is very large compared with the normal of  $\frac{1}{2}$  mm. or less. The mean pressure in veins of about 300 $\mu$  diameter shows the high figure of 7.5 cm. of water while in venous capillaries of 30-45 $\mu$  it is 10.1 cm. and in the arterial capillaries of 10-30 $\mu$ , 14.4 cm. The colloid os-

motie pressure of the blood in the species of frog used by Landis was found by White to be 9.6-11.5 cm. and, accepting these figures, we have realized, Landis points out, the conditions anticipated by Starling, involving a filtration of water from the arterial capillaries with an osmotic reabsorption into the venous. In some of the cases studied by Landis the blood pressure in practically the whole of the capillary system is in excess of the colloid osmotic pressure and a constant filtration of fluid from the blood to the lymph spaces must take place. As we shall see later on this is a normal element in the circulation of the frog.

*The hydrostatic pressure of the blood in the veins.*

In a small animal like the frog all capillary systems are practically at the same level with the heart, and the pressure is nowhere significantly increased by the weight of the blood, but in large animals the case is different. The determinations given above (p. 297) show that in the hand of a man the capillary pressure may easily rise to over 30 cm., leaving only a small margin of osmotic pressure to prevent filtration. When the hand is hanging down freely the pressure will rise further and it would appear unavoidable that in the lower part of the body the hydrostatic pressure should be in excess and filtration take place. There is, however, a mechanism which normally reduces the pressure and prevents filtration.

It will be noticed that in the chart, Fig. 76, the observed capillary pressure at the lowest level at which the hand could be held falls short a little of the hydrostatic pressure to be expected. This has been noticed before in experiments on venous pressure and is very conspicuous in the foot of man (v. Recklinghausen, 1906; Hooker, 1911), and to investigate it Miss Car-



rier and Rehberg have made some venous pressure measurements.

They determined the venous pressure in a manner similar to that employed by Recklinghausen and by Hooker, by measuring the outside pressure necessary to bring a vein to collapse. On well-filled veins this method works with an accuracy of  $\pm 1$  cm. and has no systematic errors. On veins which are already half collapsed the method is difficult to use and not very accurate.

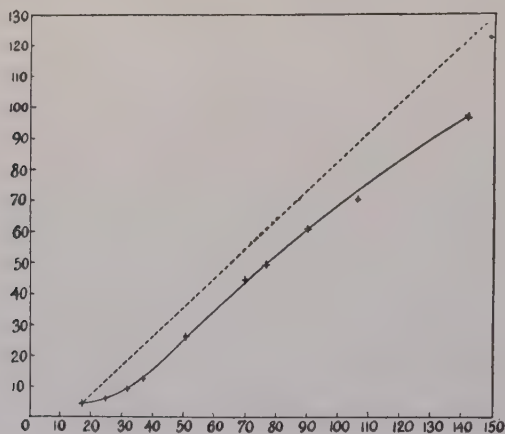


Fig. 78. Venous pressure in human foot at different levels below clavicle. The dotted line representing the theoretical hydrostatic pressure should have been drawn more to the right, probably from a point near 25 on the abscissa. After Carrier and Rehberg.

When compared with the capillary pressure the pressure in superficial veins in the hand or foot is generally lower by 2 to 3 cm. water pressure.

On a subject in a natural standing position they find, like Recklinghausen and Hooker, that the venous pressure in the foot falls very considerably short of the

hydrostatic pressure, reckoned from the lower end of the sternum about 25 cm. below the clavicle in their subject, and is, moreover, very variable. When the subject stands on one leg on a low stool and the leg to be examined hangs down freely, the discrepancy is diminished and constant results are obtained after standing for five minutes. Their chart, Fig. 78, gives a number of determinations on the subject *P.R.*, who was sitting or lying in a chair with the foot resting in different positions. In all the deeper positions the actual pressure is distinctly lower than the hydrostatic, which in the higher it exceeds slightly.

#### *The venous pump.*

This interesting phenomenon is due to slight muscular movements in the leg which the subject may feel but is unable to suppress completely, as was shown by Hooker (1911), who found, further, that when the legs were paralyzed or the subject anesthetized the pressure would rise up to the level to be expected theoretically—slightly above the hydrostatic pressure corresponding to the vertical distance below the chest.

The details of the mechanism of this “venous pump” undoubtedly deserve further study, both from the anatomical and from the physiological point of view, but, generally speaking, the pumping action is due to compression of veins by muscular movements. Owing to the valves any compression of a venous segment will, at least partially, empty that segment in the central direction, by which action the pressure is lowered and the segment can fill up again from below.

The efficacy of the venous pump is very considerable. As we have seen, even the slight involuntary movements made by a person standing in the erect position may be sufficient to reduce the pressure some 40 cm. In walking the pressure in the veins of the foot is usually

reduced to very near zero, as can be seen or felt on the veins of the foot of a walking person. In the arm of man the efficacy is much less, but even on the hand hanging down vertically the high pressure felt on palpating the veins can be considerably reduced by rapidly opening and clenching the hand a few times.

An effective venous pump is certainly present in the legs of all animals of high stature. In the hoof of the horse a special arrangement of valved veins is described (Lungwitz, 1910) which are alternately compressed and dilated in stepping movements and provide a pump which will reduce the pressure in the capillaries of the toe.

*Tissue pressure and filtration edema.*

Filtration of fluid from the capillaries to the tissue spaces is counteracted by the tissue pressure. H. Hagen (1927) believes that the pressure in the skin can be measured by the injection pressure necessary to form a wheal, but it is obvious that this idea is erroneous. What is measured is the pressure necessary to force the tissue elements apart. There is every reason to believe that in the skin as in the subcutaneous tissue the pressure is normally practically equal to the atmospheric. This holds also for the extremities below the heart, so long as no edema is present. Edema, of course, causes distension of the elastic skin and rise in pressure, counteracting the further increase of the edema. The elasticity of the skin is imperfect, however, and permanent distension is easily produced. Thus, Iversen (1928) has measured in a case of ascites an abdominal pressure of 120 mm. water. When 3 liters of fluid out of a total of 16 l. were removed the pressure was reduced to 20 mm. and fell to 0 by letting out 3 l. more. These pressure determinations were made at the uppermost point of the abdomen of patients ly-

ing flat on the back. Below this point the pressure is, of course, increased by the weight of the abdominal contents *pari passu* with the vertical distance. This is so, no doubt, in normal cases also, the contents of the abdominal cavity always exerting pressure very nearly as if they were perfectly fluid, and it follows that in this cavity the hydrostatic increase in venous pressure is exactly counterbalanced by the hydrostatic increase in tissue pressure so that, as far as the conditions of filtration are concerned, all the capillaries can be considered as being at the level of the highest part of the abdominal cavity.

When the body is immersed in water the hydrostatic pressure is everywhere counterbalanced and all the capillary systems can be considered as being at the level of the heart. This is probably of some importance for large aquatic animals such as the whales which should, therefore, in spite of their enormous size, require only a low colloid osmotic pressure in the blood to prevent filtration.

In large terrestrial animals the capillary pressure in the lower part of the legs becomes decidedly higher than the colloid osmotic pressure of the blood when the venous pump is not acting, and the occurrence of filtration edema is unavoidable. In the experiment on *P.R.*, referred to above, the subject was kept standing for fifteen minutes, during which time the circumference of the hanging foot increased by swelling from 27 to 29 cm. Some swelling of the hands is said to be frequent in soldiers having to march or stand with hanging arms for some time. Other examples of this filtration edema caused by the capillary pressure exceeding the effective osmotic pressure of the blood will be given later.

It is evident that, in spite of the activity of the venous pump, which depends wholly on contractions

of "voluntary" muscles, the capillaries in the lower parts of the body of a large animal (the udder of a cow, for instance) must often be exposed to rather high hydrostatic pressures, and it is from this point of view that the possession of blood with a high colloid osmotic pressure affords an important protection against filtration edema, the more urgently required, the higher the level of the heart is above the ground. The fact that in most large animals the heart is placed at the lowest possible level in the chest is perhaps also of some significance in this connection. Its position is, in fact, generally so low that the elephant and the giraffe are (as far as I know) the only animals now living having their heart at a higher level than man.

In the giraffe with a total height of 5 m. the heart is at a height of about 2.5 m., and it would be extremely interesting to know just how the giraffe avoids the development of filtration edema in its long legs. Unfortunately, we have not found it possible to obtain giraffe blood for determinations of the osmotic pressure.

I mentioned in my second lecture the fact that the functional capacity of an anatomical structure may depend on its size just as much as upon its form. I would like to draw your attention to the example of this rule which we have, I believe, in this case. It might be imagined that a circulatory system like that of a mammal might be reproduced in any desired dimensions, but it is at least not improbable that the giraffe is not very far removed from the limit at which, in an animal living on land, the unavoidable increase in hydrostatic capillary pressure can be compensated by increasing the colloid osmotic pressure of the blood, which, in its turn, must be limited by the consequent increase in viscosity and perhaps by other factors.

Speculations such as these, though admittedly loose,



are sometimes very useful. Sooner or later an opportunity offers of putting them to the test. It is, of course, very gratifying to find them confirmed, but generally they are even more useful when they turn out to be wrong, because, in that case, they serve to discover at what point the reasoning went astray and to guide it back into a channel which may possibly lead it onward. The problems of physiology are so complicated that, to put it tersely, one cannot expect to be able to reason correctly from the facts for more than five minutes at a stretch.

*Differences in capillary permeability.*

The statement given in the preceding lecture that normal capillaries are permeable to crystalloids and impermeable to the blood colloids is very summary and requires important qualifications. There are capillary systems in certain animals and organs which are normally permeable to colloids and most (perhaps all) capillaries can undergo reversible changes which temporarily increase their permeability.

It has been shown, notably by Starling's admirable investigations on the formation of lymph in mammals (1894), that in the liver and intestine the capillaries are normally permeable to protein to such an extent that the effective osmotic pressure becomes lower than the capillary blood pressure and a filtration of lymph through the capillary wall is constantly going on.

The most permeable capillaries are those of the liver, which fact is naturally correlated with their very peculiar structure as referred to in my fourth lecture.

The normal blood pressure in the liver capillaries is extremely low, as inferred by Bayliss and Starling (1894) from simultaneous determinations of pressure in the portal vein and the cava, and the flow of lymph is, therefore, not very great. Any rise of pressure in

the liver capillaries will, however, bring about an increase in the lymph flow proportional to the pressure, and the composition of this lymph will approach so near to that of the blood plasma that it must be concluded that the capillaries are permeable to all the blood colloids. The filtration of the colloids is, however, undoubtedly slower than that of the crystalloids, and this will cause some dilution of the lymph, as compared with the plasma, and check the flow to a certain extent.

The capillaries of the intestinal mucous membrane are also permeable to protein, but the protein content of the intestinal lymph is always lower than that of the blood and in the light of the experiments on fractional osmotic pressure, mentioned in the preceding lecture, it seems natural to assume that the capillaries in question are permeable to a certain fraction of the blood protein. This fraction appears to exceed one-half or perhaps two-thirds of the total protein by weight, and, since it must be the smaller molecules which can pass out, the resulting reduction in the effective osmotic pressure of the blood will be even greater. Bayliss and Starling (1894) have found the pressure in the portal vein of medium-sized dogs to be about 100 mm. of water. The pressure in the intestinal capillaries must be higher, but the difference is probably small. The available information is consistent, however, with the assumption that there is normally in the intestine some excess of capillary pressure over the effective osmotic pressure, which explains the observed constant production of lymph.

Starling has, by his varied experiments, established the fact that any increase in pressure in the intestinal capillaries brings about a corresponding increase in the flow of lymph from the intestine, and at the same time he has verified the older observation that, during

any such increase, the percentage of solids (that is, of protein) in the lymph is diminished.

This observation is of some theoretical importance. Generally speaking, the rate of lymph filtration must be determined by the rate of filtration of the slowest substance. If we suppose, for instance, that water is filtered at a more rapid rate than the salts, the excess of water in the filtrate would set up osmotic forces, counteracting the filtration of water and reducing its rate to that of the salts. Hence it follows that the proportion of crystalloids in the filtrate must be the same as in the blood. The osmotic force set up by a protein deficit in the lymph is so low, however (about 50 mm. water pressure for 1 per cent of protein, according to the determinations given in the preceding lecture), that it may be overcome by the filtration pressure, and this is obviously what is going on in the intestine when lymph is produced by filtration at a rapid rate.

It is highly probable that the permeability to colloids demonstrated for the liver capillaries and those of the intestinal mucous membrane in mammals exists also in the corresponding organs in the other classes of vertebrates, and in addition there is in frogs and toads, and probably in all amphibia, a further normal mechanism by which large amounts of fluid are filtered off from the blood and returned to it via the lymph channels. I have to remind you that in these animals we have two portal systems, including the veins not only of the intestines, but also of the main part of the skin and the hind limbs generally. The capillary pressure in these regions is comparatively high owing to the second resistance in the kidneys and liver. We have, further, extensive and intercommunicating lymph spaces between the skin and the muscles in the batrachians, and finally we have in all amphibia four

lymph hearts which take up fluid from the subcutaneous spaces and return it to the blood. In Brückes' laboratory Isayama (1924) and later Ito (1926) made the experiment of stopping the inflow of lymph to the blood by cauterizing or curarizing the lymph hearts and observed a rapid increase in the concentration of the blood, reaching about 30 per cent in 10-20 minutes. The exudation of fluid from the blood deduced from the observations of Isayama amounts to at least the weight of the animal in 24 hours, and more probably double that quantity.

According to the determinations of Landis (1926) the average blood pressure in the mesenteric venules of the frog is 75 mm. water, and the veins of the two portal systems are connected up in such a way that the pressure in the capillaries of the hind legs and most of the skin cannot be lower than this. In European frogs the colloid osmotic pressure of the blood is often below this figure and a steady filtration of water and crystalloids is, therefore, to be expected in all such cases, even if the capillaries were impermeable to colloids. The determinations made in my laboratory by Churchill, Nakazawa, and Drinker (1927) show, however, that some capillaries at least must be permeable to protein, since the fluid collected from the lymph spaces contains protein in a percentage from 0.3 to above 2 (usually somewhat over 1 per cent) and with a colloid osmotic pressure corresponding to the protein content and being on an average 42 mm. water. The experiments of Landis, presently to be discussed in detail, show that the capillaries in the frog's mesentery are certainly impermeable to protein; with regard to the muscle capillaries we have no definite evidence one way or the other, but I think it possible that they too are normally impermeable. In the determinations of Drinker and his collaborators the lymph

samples were usually taken just above the foot and must be mainly derived from the skin and web and it is natural to infer that the skin capillaries are responsible for the protein content.

Summing up the evidence of the present and of the preceding lecture concerning the relation between the capillary pressure and the effective osmotic pressure of the blood we have to admit that it is too fragmentary to allow any general conclusions. In one case, that of the human skin, we find the effective osmotic pressure considerably in excess of the capillary blood pressure; in another, the frog's mesentery, the capillary pressure is raised practically to the level of the effective osmotic pressure, while in the skin of the frog, the liver and intestinal mucous membrane of mammals, the effective osmotic pressure is lowered by the permeability of the capillaries to some or all of the blood colloids leaving the capillary blood pressure in excess.

There is indirect evidence, however, showing that the case of the human skin is typical for a large number of tissues, namely, the fact demonstrated by Lewis (p. 165) that in the human arm the capillary pressure can be increased by venous congestion to 25 mm. mercury (= 340 mm. water) before any gradual increase in volume of the arm, indicating filtration of fluid into the tissue spaces, will take place, and further the ease with which colloid free fluids are completely absorbed into the blood vessels from the subcutaneous tissue, muscles, and the peritoneal and pleural cavities. The pleural absorption is of special significance since, owing to the "negative" pressure, fluid present in the pleurae cannot escape except by such absorption.

It is significant, finally, that in normal animals the blood can be considerably diluted by infusion of saline (Magnus, 1899) without any resulting exudation, and



exudation is absent also in some human cases where the protein content of the blood is abnormally low, as we shall see in the last of these lectures.

*The exchange of water against diffusible substances.*

The statement that normally the blood will in most tissues attract water from the tissue spaces fails to take into account the movement of water in working muscles and secreting glands.

In their beautiful researches on the "effects of functional activity in striated muscle and the submaxillary gland," Barcroft and Kato (1915) have measured the exudation from the blood in these organs in dogs by the ingeniously simple method of measuring the blood flow and comparing the hemoglobin percentages in the arterial and venous bloods, respectively. An increase in Hb. percentage, brought about by the passage through an organ, means, of course, a corresponding concentration of the blood and thus measures the quantity of fluid given off to the organ. The production of lymph, which they found to be insignificant in the resting gastrocnemius muscles and nil in the gland, becomes very considerable during, and for hours after, activity. In muscle the enormous exudation of 5 cc. per 100 g. muscle per minute has even been recorded for a short period, though in most cases it did not exceed 2 cc. Even this means that a volume of lymph equal to that of the organ is produced in less than one hour. In the secreting submaxillary gland most of the fluid given off from the blood normally goes to form saliva, but a flow of lymph takes place also and may reach the same rate per minute as the saliva.

The mechanism of this lymph production during activity is probably complex and may vary according to the organ, but the fact that it is closely bound up with a large increase in the rate of oxidation suggests that

it may partly, at least in muscles, be caused by metabolic products, which diffuse sufficiently slowly to be for a time osmotically active. Most organic crystalloids including glucose diffuse so slowly compared with water that an increase in their concentration outside the capillary wall will attract water from the blood.

As a striking example of this influence I would mention the pulmonary edema produced experimentally by Laqueur (1919) by the injection of 1 cc. of concentrated (50 per cent) glucose solution into the trachea of rabbits. The sugar attracts water osmotically from the blood in the pulmonary circulation; diffusible salts—NaCl in particular—pass out also at the same time that the sugar is passing slowly inward. In less than an hour the quantity of fluid in the lungs has increased to 15 cc. or more and has become isotonic with the blood, whereupon the volume begins to decrease by absorption into the blood.

In numerous experiments by Brasol (1884), Leathes (1885), White and Erlanger (1920), and others, glucose solutions have been injected into the blood of animals with the invariable result that water is drawn from the tissues into the blood at such a rate that the normal osmotic pressure (total osmotic pressure) is reestablished in one-half to two minutes, whereupon there is a comparatively slow return to normal conditions, as the surplus sugar diffuses out into the tissues and is excreted by the kidneys.

In an interesting series of experiments Clark (1921) has studied the absorption of nearly isotonic solutions of various substances into the blood from the peritoneal cavity of rabbits. He finds that the rate of absorption of the fluid as a whole depends upon the diffusibility of the dissolved substance, becoming slower with diminishing diffusibility. Glucose diffuses

so slowly, compared with the salts, that the amount of fluid in the peritoneum is actually increased during the first couple of hours, because salts will diffuse out from the blood and draw the necessary water to maintain the isotonicity of the fluid. After three hours about 75 per cent of the glucose is absorbed, but, even at this time, the volume of fluid present may be in excess of the volume injected.

Clark draws from his experiments the very interesting conclusion that the membrane in question (capillary endothelium + peritoneal epithelium) does not show any selective permeability. The relative rates at which the different substances pass through are, at least approximately, the same as the rates at which they diffuse through dead membranes and even through gelatine or water.

## LECTURE XIV

### CHANGES IN CAPILLARY PERMEABILITY AND THEIR MECHANISM

**T**HE permeability of the capillary wall can be increased by a number of stimuli. Except in a single case to be referred to later on in the present lecture we have no evidence that it can be diminished below the normal, but a priori it does not seem unlikely and the possibility should be kept in mind.<sup>1</sup>

I described in the first lecture the peculiar condition, called true stasis, sometimes observed in capillaries exposed to any kind of injury and easily brought about experimentally in the frog's tongue by the application of urethane. In this condition the plasma is filtered off completely from the corpuscles through the capillary wall, and we must infer, therefore, that the endothelium has become permeable to all the plasma colloids. When stasis occurs in spite of a free venous outflow the filtration of plasma is evidently very rapid, but is brought to a standstill when the capillary in question is completely filled with packed corpuscles. A less rapid filtration can be continued over a long period and leads to local edema.

Local edema in the form of wheals and blisters is easily produced in the human skin and has been extensively studied by Thomas Lewis. As fully set forth in a preceding lecture (X) Lewis could show that the whealing resulting from the most varied stimuli is due to the formation in the tissue of an H-substance allied

to histamine. The wheal fluid, as sampled by Lewis, clots readily which shows it to contain the least diffusible, probably, of the plasma proteins—fibrinogen. By an ingenious method of comparison Lewis has succeeded in estimating, on the fraction of a mm.<sup>3</sup> available, the protein content of wheal fluid and found it to be some 70 to 80 per cent of the corresponding blood serum, which again shows the capillary wall to be permeable to all the plasma proteins.

The rate of development of local edema is very variable and depends, when the walls are completely permeable, partly on the blood pressure in the vessels concerned, but, as Lewis has shown (p. 73), mainly on the rate of blood flow through these vessels. Lewis makes the following very instructive calculation: In the formation of a wheal the skin thickness is sometimes doubled over the wheal area within three minutes. If we assume that 50 per cent of the blood transudes this means a blood flow equal to the original skin volume in  $1\frac{1}{2}$  minutes or 7-14 times that measured for the human arm during rest. In many cases, of course, the whealing progresses much more slowly.

The increased permeability leading to local edema can be produced through nerve stimulation as shown by Bruck (1909) who stimulated one glossopharyngeal nerve in the frog and saw a pronounced edema develop in the part of the tongue innervated. The wheals and blisters developing in the areas innervated from the affected ganglia in herpes zoster show that a corresponding mechanism exists in mammals, though here exudation has not, so far, been produced experimentally through nerve stimulation.

When I began considering the mechanism of the increase in permeability now described I was led at first, judging from the rapidity with which fluid leaves the capillaries and by a possible analogy with the diapede-



desis of red corpuscles, to assume that real openings were formed in the capillary wall, and I formed the hypothetical conception of the formation of fissures *between* the endothelial cells which might occur, I thought, especially at points where the borders of three or more cells meet. This conception proved to be quite erroneous when put to the following test.

Dialyzed and filtered Indian ink, the particles of which are submicroscopical and can be taken to be on an average about  $200\mu\mu$  in diameter, while many of them must be smaller, is added to the blood so as to make the plasma in the capillaries of a distinct gray color, while in the larger vessels it is almost black. If microscopic openings were now formed in the capillary wall by the application of urethane the gray plasma would be filtered off, but the result of the actual experiment was that the Indian ink particles were held back quantitatively while the clear plasma disappeared as before. This experiment has been repeated and varied in several ways, always with the same result.

Discarding this idea I came to the conclusion that mechanical stretching of the capillary wall must be responsible for the increase in permeability regularly seen to accompany dilatation, and this was the view maintained in the first edition of this book. While definitely admitting the possibility that the permeability of endothelial cells can become altered independently of any change of caliber I could not accept as conclusive the evidence for such alterations then available. This skeptical attitude is also justifiable toward much of the later evidence, but the situation has now been wholly changed through the brilliant work of E. M. Landis (1927-1928, I-III). I insisted in 1922 upon the necessity for a quantitative formulation of the permeability problems and "quantitative even if roughly approximate, determinations of the properties of the

capillary wall." We wanted, I said, to know the absolute permeability of capillaries, the size of molecules which they will let through in different conditions, and further, and just as badly, to obtain some information about their filtering capacity for water. While I had made a feeble beginning to measure absolute permeabilities by means of colloid dyes I despaired of finding means to measure the rate of filtration. The re-

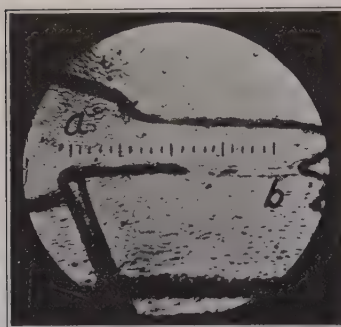


Fig. 79. Capillary in frog's mesentery 2 minutes after stoppage of flow at *a*. Filtration of fluid. Capillary pressure 26 cm. of water.  $\times 80$ . After Landis.

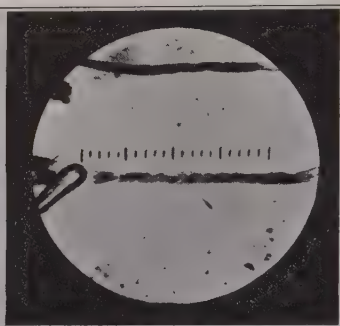


Fig. 80. Another capillary similarly treated. No change in position of corpuscles. Pressure 9.5 cm.  $\times 70$ . After Landis.

searches of Landis have now shown that this problem can be successfully attacked, and information of the greatest value has already been obtained in the new field.

Landis exposes the mesentery of a large frog and keeps it under irrigation with frog Ringer. By applying a gentle pressure with a microscopic glass rod he stops the flow in a suitable capillary. If the blood pressure in the capillary is in excess of the colloid osmotic pressure of the blood the corpuscles in the closed capillary will be observed to move toward the glass rod

as in Fig. 79; if there is equilibrium they will remain stationary (Fig. 80), and if the colloid osmotic pressure is in excess they will move out of the capillary. The blood pressure is measured by Landis' micro-injection method and the rate of movement of a suitable

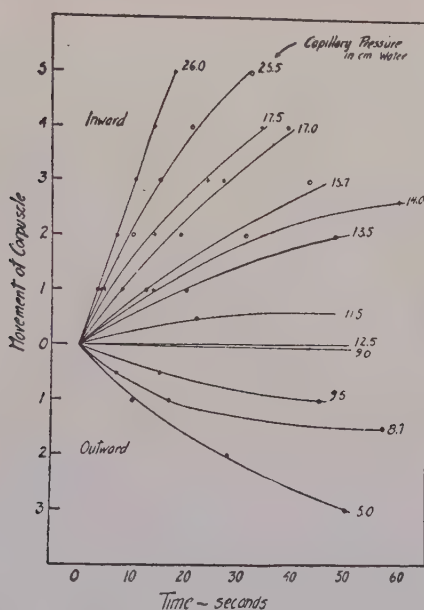


Fig. 81. Rates of movement of single corpuscle in closed capillary as determined by capillary pressure and time. After Landis.

corpuscle is read off on the micrometer scale shown in the figures. The rate of movement becomes gradually slower as illustrated in Fig. 81, because the protein concentration is altered by exudation or absorption of water, but from the curves it is possible to deduce the initial rate. When the diameter of the capillary and the distance of the observed corpuscle from the glass

rod are also measured the rate of filtration in cubic  $\mu$  per square  $\mu$  per second can be calculated. In Fig. 82 Landis has given a large number of such measurements. The observations are arranged with the capillary pressure as abscissa, and it is evident that they can be fairly well represented by the straight line

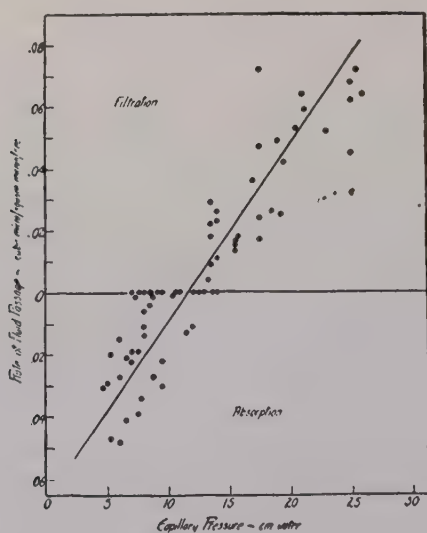


Fig. 82. Initial rates of filtration and absorption as determined by capillary pressure.  
After Landis.

drawn. This line shows that on an average neither absorption nor filtration will take place at a pressure of 115 mm. of water which should, therefore, correspond to the colloid osmotic pressure of the blood in the capillaries.

When the rate of filtration is calculated per unit pressure (cm. of water), unit area (sq.  $\mu$ ), and unit time (second), Landis finds no systematic difference

between wide and narrow capillaries. He points out that when the mesentery is first exposed all the capillaries are narrow. The exposure causes a general dilatation, from which some capillaries recover and contract, while others remain dilated. These experiments and a further series in which the filtration of perfused dye solutions has been studied (see p. 327) show, therefore, that the permeability of these capillaries as expressed by their filtering capacity for water is independent, within the limits studied, of their state of dilatation.

Landis shows further that the rate of filtration is definitely increased by injury to the capillary wall. In one series of experiments he irrigated the mesentery with 10 per cent alcohol in Ringer before measuring the rate of filtration. This caused the blood flow to become more sluggish, while the corpuscles became concentrated, indicating qualitatively an increase in filtration and permeability to the plasma colloids. Measurements of the filtration in single capillaries showed the rate to be increased sevenfold while the point of equilibrium (the effective osmotic pressure) appeared to be reduced from 115 to about 40 mm. Similar experiments, in which mercuric chloride 1-10,000 was added to the irrigation fluid, gave an identical result (Fig. 83).

In his latest contribution (1928) Landis has studied by essentially the same method the effects of oxygen lack, increased  $\text{CO}_2$  pressure, and increased acidity. Irrigating the mesentery alternately with aerated and oxygen-free Ringer and measuring the rate of movement of a single corpuscle in a closed capillary he obtained the extremely interesting curves reproduced in Fig. 84. At pressures which are fairly close to the normal colloid osmotic pressure (13.3 and 8.7 cm., respectively) the corpuscle in the experiments with



oxygenated Ringer soon comes to a standstill, because equilibrium is obtained by dilution or concentration of the plasma. In oxygen-free Ringer this equilibrium becomes disturbed after 30-50 seconds and a rapid

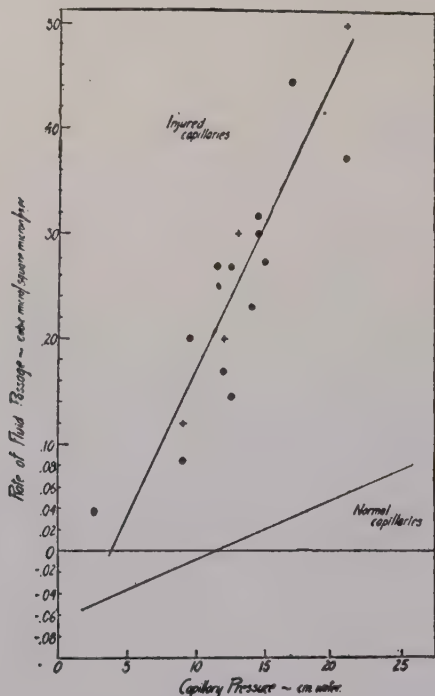


Fig. 83. Rates of filtration from capillaries treated with alcohol or mercuric chloride as determined by pressure. After Landis.

filtration of plasma begins, indicating injury to the capillary wall. In order to obtain quantitative results Landis found it necessary, further, to stop the blood flow to the mesentery for three minutes, because otherwise oxygen would diffuse into the capillary under observation from the adjacent vessels in which blood

carrying oxygen was freely circulating. Special control experiments showed that this procedure causes only a slight increase in permeability when the mesentery is bathed in oxygenated Ringer. After resumption of the blood flow a filtration experiment was made on a single capillary.

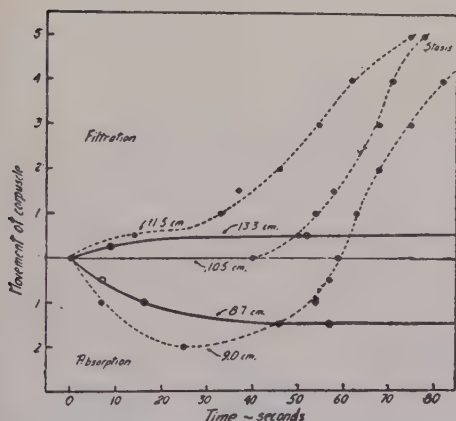


Fig. 84. Rates of movement of single corpuscles in closed capillaries determined by capillary pressure and time. Full line curves aerated Ringer. Broken lines oxygen free Ringer. After Landis.

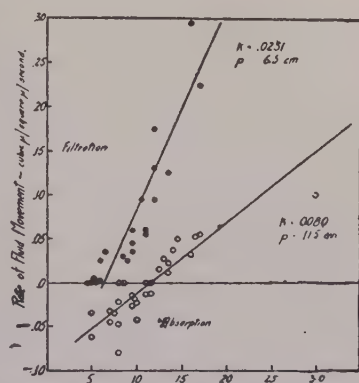


Fig. 85. Initial rates of filtration from capillaries deprived of oxygen, and after 15 minutes recovery. After Landis.

The results of 70 such experiments are summarized in Fig. 85. After deprivation of oxygen for 3-4 minutes the capillaries show an effective osmotic pressure of 65 mm. and a rate of filtration increased about fourfold. With oxygen a rapid recovery takes place; a normal osmotic pressure is often reached in 3-5 minutes. In all cases the determination was repeated 15 minutes later, and the results are also given in Fig. 85 as small circles. The osmotic pressure is now normal and the rate of filtration only slightly increased.

Stoppage of blood flow for 5-10 minutes in the absence of oxygen usually increases the permeability to such an extent that an irreversible stasis develops when blood is again admitted, as with 10 per cent alcohol.

Special experiments were made to test the (extremely remote) possibility that the change might be due to accumulation of  $\text{CO}_2$  or acid metabolites generally. Various concentrations of  $\text{CO}_2$  up to half saturation which reduced the  $p_{\text{H}}$  from 8.2 to approximately 5.4 produced no measurable change in the rate of fluid movement or in the effective osmotic pressure, though the mesenteric circulation was to some extent modified by dilatation of the capillaries and a more rapid flow of blood through the capillary network. Ringer solution fully saturated with  $\text{CO}_2$  ( $p_{\text{H}}$  5.1-5.2) produced a slight increase in the rate of filtration from the normal 0.0056 to 0.0088, but no change in the effective osmotic pressure. Solutions made acid by the addition of  $\text{HCl}$  gave the following results.

$p_{\text{H}}$ of Ringer's fluid	Number of observations	Filtration constant	Effective osmotic pressure cm. water
8.0	70	0.0056	11.5
6.0	58	0.0065	11.7
5.0	28	0.0074	11.4
4.0	28	0.0152	11.6
3.5	22	0.0207	7.8
3.0	Rapid stasis		

There is a gradual but slow increase in filtering capacity with increasing acidity up to the point where the very acid solutions definitely injure the endothelium. It is evident that changes in  $p_{\text{H}}$  within physiological limits have no effect whatever.

It is interesting to compare the filtering capacity found by Landis for normal endothelium with that

determined for artificial collodion membranes which are, like endothelium, impermeable to plasma protein. By multiplication with 600 the filtration constants given above are transposed to the units employed in the preceding lecture (cc. per minute through 100 cm.<sup>2</sup> at the pressure of 1 atmosphere).

This gives for the normal endothelium a filtering capacity of 3.4 which can be raised to 9.1 before the endothelium becomes permeable to protein. Our normal membranes have a filtering capacity of 1 which can be raised to 1.4, but it must be remembered that they are more than a hundred times thicker than the endothelium.

I cannot refrain from expressing a slight doubt with regard to the absolute accuracy of Landis' figures. The rate of filtration was measured by observation of blood corpuscles, and it seems to me that, at the very slow rates of movement concerned, these might lag behind and the plasma movement be more rapid. I have to admit on the other hand that such a lag would probably be less at the fastest rates measured and thus militate against the possibility of expressing the results by straight line curves. There is the further difficulty that the colloid osmotic pressures determined by the point of equilibrium in Landis' curves appear to be rather high, and this applies with especial force to the pressure of 40 mm. determined for the capillaries treated with alcohol or mercuric chloride. These capillaries go into complete stasis, filtering off the whole of the plasma. With an effective osmotic pressure of 40 mm. a considerable proportion of the plasma should be left in the vessels at any of the pressures investigated, and the close packing of corpuscles necessary to produce stasis with a free venous outflow would be impossible.

In spite of these doubts with regard to the absolute

accuracy of individual results it is evident that Landis' methods of quantitative study open up the possibility of an intimate understanding of capillary physiology far beyond anything to be imagined before. I hope they will be pushed vigorously and extended to other capillary systems to minimize the dangers of too hasty generalizations. It cannot be emphasized often enough that the capillaries of separate tissues differ greatly in their reactions. As a case in point I would refer to the skin capillaries (and venules) of man which are, according to the experiments on reactive hyperemia discussed in Lecture X, much less susceptible to damage by lack of oxygen than those of the frog's mesentery.

*The absolute permeability of capillaries.*

I have defined the absolute permeability by the size of the particles or molecules which a membrane will just let through, and an attempt was made in my laboratory some years ago to measure absolute permeabilities of capillaries in varying states. We found that highly colloidal dyes like brilliant vital red or Chicago blue 6B would diffuse very slowly through the normal capillary wall in the frog's tongue or web, while in all places where capillaries were dilated they would become surrounded by a stained zone.

A few experiments have been made with soluble starch, the particles of which are said to be about  $5\mu\mu$  in diameter. Starch is held back by normal capillaries. It passes out when they are strongly dilated and can be detected by the addition of a dilute iodine solution. The size of the pores in this latter case is, therefore, above  $5\mu\mu$ , while our experiments with Indian ink show that they are below  $200\mu\mu$ .

The peculiar difficulties inherent in determinations of this kind were not realized when I did my experi-



ments, but have been brought out clearly by some of Landis' experiments (II, 1927). He perfused single capillaries in the frog's mesentery with dye solutions, selecting a small number of dyes with increasing colloidal properties from vital red HR (= trypan red) through toluidine blue and trypan blue to brilliant vital red and noted the time from the beginning of the perfusion until the dye became visible on the outside of the perfused capillary. All these dyes, with the possible exception of brilliant vital red, are able to pass readily through the normal capillary wall, but Landis shows that the rate at which they pass is determined by the pressure in the perfused capillary. This means, of course, that the dye molecules do not pass (mainly) by diffusion—that is, by reason of their individual Brownian movements—but are carried through by the filtration current of water. It is, therefore, not the absolute permeability, but the rate of filtration of water which is approximately measured. Landis gives the very instructive charts, Figs. 86-89. The first two show the relation between rate of filtration (as indicated by toluidine blue) and the capillary diameter and pressure, respectively, demonstrating again that filtration is essentially independent of the state of dilatation. The last two show the relative rates of passage of the dyes studied in living and artificial capillaries, respectively. It is to be noted that the dye with the smallest molecules, vital red HR, diffuses so rapidly through the living capillary wall that its time of appearance on the outside is practically independent of the pressure. This illustrates the very important point emphasized before (p. 275) that the exchange of crystalloids is practically independent of any flow of water through the capillary wall. The much slower passage of the same dye in negatively charged collodion capillaries is probably due, as pointed out by Landis, to its acid prop-

erties, while acid and basic dyes will apparently pass indiscriminately through living capillaries. It is to be concluded generally from Landis' experiments that the failure of a dye to become visible within a reasonable time on the outside of a capillary does not necessarily mean that the capillary wall is impermeable to the sub-

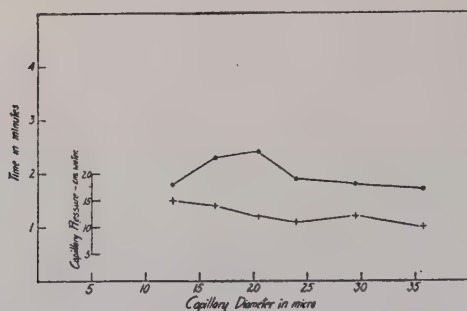


Fig. 86. The relation between capillary diameter and the rapidity with which 0.015 M toluidine blue passes through the wall. After Landis.

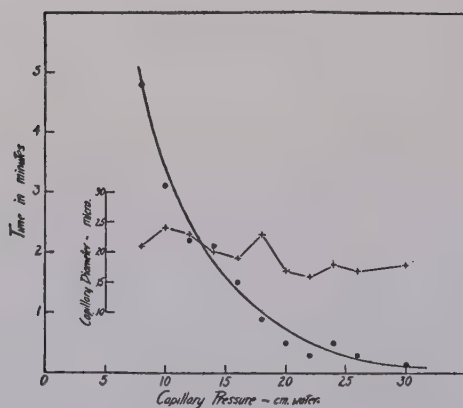


Fig. 87. The relation between capillary pressure and the rate of passage of toluidine blue. After Landis.

stance, but may be due to an insufficient filtration of water. While it is necessary, therefore, to exercise the utmost caution in drawing conclusions regarding absolute permeability from experiments with dye substances I think I can show you that information of considerable interest and value can nevertheless be obtained.

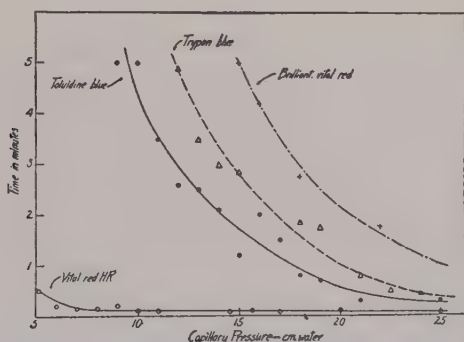


Fig. 88. The relation between capillary pressure and the rate of passage of different dyes in equimolar concentration. After Landis.

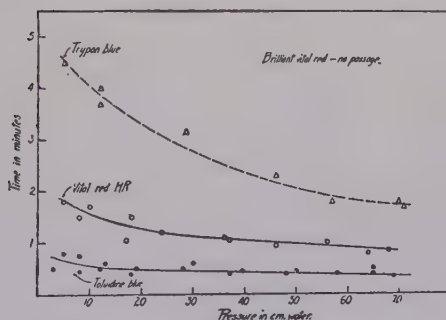


Fig. 89. The relation between perfusion pressure and the rate of passage of different dyes through artificial collodion capillaries. After Landis.

Florey (1925) succeeded in proving that, like crystalloids, colloids do pass directly through the cytoplasm, but not through the nuclei, of endothelial cells. Perfusing inflamed tissue with a solution containing soluble starch and immediately afterward with formaline containing iodine he precipitated the blue starch iodine compound within the endothelium.

Herzog (1925, 1) in experiments on the frog's tongue introduced Chicago blue and Indian ink together into the circulating blood. Any stimulus causing dilatation (mechanical, thermal, or chemical) brought about a passage of dye, while at the same time the endothelium was covered with a layer of Indian ink particles which appeared to obstruct any further passage of dye. In some cases the Indian ink was taken up later on by the endothelial cells.

Hoff and Leuwer (1926) have experimented on human subjects injecting Congo red intravenously. This dye remains for a long time in the circulation, but they find that in inflamed tissue or tissue undergoing amyloid degeneration the dye leaves the vessels rapidly. If fluid (0.1 cc. saline) is introduced intradermally, Congo red is given off from the blood into the artificial wheal so formed. The quantity is very small when the injection is practically painless (Ringer, isotonic NaCl or  $\text{CaCl}_2$ ), larger with  $\text{MgCl}_2$  which causes some pain, and considerable with KCl which gives much pain. An intradermal injection of serum gives rise to some pain and becomes strongly colored. This result, though at first sight conflicting with the evidence just given, is easily explained from Landis' experiments. The capillaries even in the normal state are not impermeable to Congo red, but the diffusion is too slow to cause a visible extravascular coloring. When a filtration current is set up either by injury to the vessels, by which they become permeable to protein, or because the colloid

osmotic pressure is counterbalanced by colloids outside the vessels, the filtration of water will carry the dye through in sufficient concentration.

In a later paper Hoff (1927) describes experiments on an urticaria factitia patient. He shows that a wheal can be raised by stroking even in a place where the minute vessels are contracted by adrenaline and that Congo red will appear in such a wheal. He finds that when a wheal has just subsided and also in fields stimulated with mustard, or otherwise, a renewed stimulation of the same spot will fail to raise a wheal. This is the phenomenon described and repeatedly referred to by Lewis and his collaborators (Lewis, p. 1240) as refractoriness and believed to be due to an alteration of the vessel wall which becomes for a time impermeable, although stimulated and dilated by the H-substance. Strange to say Lewis' work is unknown to Hoff who proceeds to explain the observation along a very different line. By giving the second stimulus after an intravenous injection of Congo red he shows that the "refractory" vessels are freely permeable and that there is, in fact, a condition of "latent" edema in the area which fails to wheal. The elastic properties of the skin in question are shown to be altered, the texture of the skin is loosened, and numerous fissures are supposed to be opened up through which the exuding fluid flows off more or less rapidly into the subcutaneous spaces. I accept unreservedly the observations and interpretations given by Hoff. They explain as far as I can see all the observations on refractoriness brought forward by Lewis, and they explain further the puzzling phenomenon studied by Goldscheider and Hahn (1925) and utilized as a clinical test (Guggenheim and Hirsch, 1926; Cohen, Applebaum, and Hainsworth, 1926) that an artificial wheal produced by the intradermal injection of 0.1 cc. saline which is very slowly absorbed from normal skin dis-



appears at a much more rapid rate when the skin shows latent (Oedembereitschaft) or manifest edema. In these cases only a little of the fluid is directly absorbed into the blood vessels while the rest flows off into the subcutis.

*The mechanism of increased permeability.*

It will be convenient at this stage to summarize the known facts about increased permeability and to correlate them with the results regarding changes in capillary diameter given in preceding lectures. This is attempted in the adjoined tabular diagram.

Animal	Organ	Stimulus	Effect on capillary diameter   permeability		Symptoms observed
mammals	skin	nervous	increase	not observed	edema
frog	tongue	nervous	increase	increase	
frog	web	nervous	increase	not observed	
man	skin	herpes zoster	increase	increase	
man	skin	cold (paralysis)	increase	not observed	wheals and blisters
frog	tongue and others	urethane	generally increase	increase	
mammals	skin	ether, chloro- form, and other narcotics	increase?	increase	subcutaneous edema
mammals	skin	arsenic, phos- phorus (capil- lary poisons)	increase?	increase	subcutaneous edema
frog	tongue	mechanical	increase	increase	stasis
frog	tongue and others	histamine	nil	nil	
man and mammals }	skin and others	histamine	increase	increase	whealing
man and mammals }	skin and others	H-substance from injury	increase	increase	whealing
man and mammals }	skin and others	oxygen lack (H-substance?)	increase	doubtful or nil	increased filtration and stasis
frog	mesentery	oxygen lack (H-substance?)	doubtful or nil	increase	
frog	tongue	light (H-substance?)	increase	increase	
man	skin	light (H-substance)	increase	increase	whealing and blistering

Most of the instances given in this table have been discussed in this and preceding lectures but with regard to some of them it is necessary to add a few words.

In some very interesting experiments Magnus (1899) studied the effect of plethora induced by infusion of saline into the blood. He found that in normal animals (dogs and rabbits) the blood can be considerably diluted without the production of subcutaneous edema, showing the existence of a certain reserve of colloid osmotic pressure in the normal blood. In animals a half hour dead, the infusion produces a very pronounced edema, and the same is the case in living animals which have been treated with arsenic or phosphorus or are deeply anesthetized with chloroform, ether, or chloral. Magnus mentions also some experiments by Cohnheim and Lichtheim, in which a very slight experimental inflammation gave rise to a local edema when the blood was diluted by infusion of saline.

These experiments show that an increase in permeability which is too slight to give rise to symptoms so long as the blood is normal will do so when the reserve of colloid osmotic pressure is sufficiently reduced by dilution of the blood. The evidence for capillary dilatation resulting from the action of these substances is indirect and inconclusive. Certain narcotics (urethane) and capillary poisons (gold salts) have been seen microscopically to produce dilatation, but for those here dealt with it has been inferred mainly from their aggravating action in shock, which may be due exclusively to the increase in permeability.

It has been observed in numerous experiments by different investigators that the capillary dilatation caused by local mechanical stimulation may give rise to considerable exudation of fluid. I have seen it some-

times in the frog's tongue when a sharply localized dilatation was brought about by repeated very weak stimulation with a hair. Ebbecke (1917, p. 31) has seen an evanescent edema of the liver surface in mammals accompany the red reaction produced by slight mechanical stimulation.

In certain tissues the permeability appears to be increased without any dilatation. Landis has shown that this is the case in the frog's mesentery under treatment with urethane (I, 1927) and mentions also the case of compressing a capillary in the same tissue with a glass rod and thereby causing subsequent filtration of plasma and dye through the damaged cells. A general symptom of the injury is that the cells become sticky so that foreign particles and even the red corpuscles will adhere to them.

Injury may be brought about directly or indirectly. In the case of the frog's mesentery, injured mechanically or by urethane, alcohol, or mercuric chloride, we have no reason to doubt the direct action on the capillary wall of the injurious agent. In many other cases, especially in mammals, injury acts through the liberation from the injured tissue cells of a special H-substance which acts both on the Rouget cells and the endothelium, causing dilatation and increase in permeability. We may very probably have H-substances acting also in the frog, but they must be different from those of mammals since the prototype of the mammalian H-substance, histamine itself, causes neither dilatation nor increase in permeability (Herzog, 1925, 1), when applied to frog capillaries.

Lack of oxygen is a special form of chemical injury. It has been shown to act in the human skin and in mammals generally through the formation of a special substance possibly related to, but certainly not identical

with Lewis' H-substance of gross injury. It causes in a number of mammalian tissues a prompt dilatation of capillaries and somewhat larger vessels, but appears to have little if any influence upon permeability. No subsequent edema is brought about by occlusion of an arm for many minutes. In the frog's mesentery, on the other hand, lack of oxygen causes a prompt increase in permeability, very definite after 30 seconds' exposure and leading to irreversible stasis after less than 15 minutes while the action on capillary diameter appears to be insignificant.

While the table shows a general parallelism between capillary dilatation and increased permeability the cases now discussed show that this parallelism is not complete. There are notable exceptions and we have to admit, therefore, that the mechanisms are probably different. The diameter of capillaries is governed mainly—perhaps exclusively—by the tonus of the Rouget cells while the permeability depends upon the state of the endothelium. The endothelium may become increasingly permeable and even completely permeable to all the blood proteins without being stretched. Some dilatation may take place without any increase in permeability, but I find no case in which a considerable dilatation has taken place without being accompanied by an increase in permeability. Mechanical stretching of the endothelium takes place only when dilatation proceeds beyond the smoothing out of the endothelial folds characteristic of a contracted capillary and it appears possible, therefore, that mechanical stretching is one cause of increased permeability. If this should turn out on renewed experimentation not to be the case we must accept the remarkable coincidence between increase in diameter and increase in permeability shown in the table as wholly fortuitous.

*Restoration of normal impermeability—A permeability hormone?*

The increase in permeability brought about by the stimuli discussed is usually reversible. Even in cases where stasis has developed the column of corpuscles is sometimes broken up and normal flow resumed. A necessary condition for such resumption is that the normal impermeability to the plasma colloids is restored. The processes responsible for this restoration are little known, being more obscure even than the processes leading to increase in permeability.

It has been repeatedly observed by a number of investigators that calcium salts, administered subcutaneously or per os, may diminish exudation in inflammation (Chiari and Januschke, 1910), retard the absorption of dyes from tissue spaces and their passage from the blood into the aqueous humor (Rosenow, 1916), and diminish exudation into the lungs after phosgene poisoning (Laqueur and Magnus, 1921). All these effects are commonly ascribed to an effect of the increased calcium content of the blood upon the capillary endothelium, which is supposed to be rendered less permeable. This may be so, but the evidence so far brought forward can scarcely be accepted as conclusive. Perfusion experiments have been made by R. Hamburger (1922) to study this problem, but, as far as I understand them, their results seem rather conflicting.

In some of their experiments Hoff and Leuwer administered  $\text{CaCl}_2$  with Congo red but failed to find any evidence of reduced permeability, and the same may be said of the experiments of Descamps (1925).

In 1921 Ellinger and Heymann published a series of perfusion experiments on the hind legs of frogs in which they attempted to estimate quantitatively the rate at which edema would develop. Their perfusion



fluids were partly Ringer solutions with various crystalloid additions, partly mammalian serum and mixtures of serum with crystalloids. Their results could not be utilized, because they paid no attention to the condition of capillaries, but the fact remained that serum appeared to have an effect in preventing or reducing edema out of proportion to its colloid osmotic pressure. This clue was followed up by Drinker (1927) in my laboratory.

Drinker made perfusions of one foot of a frog (*R. temporaria*) arranged in such a way that swelling and shrinking of the web could be estimated directly by the fine-adjustment of the microscope. The state of dilatation of the web vessels could be photographed at every stage by changing for a brief period to a perfusion fluid containing graphite ink which by virtue of the small size of the particles will fill the capillaries completely without leaving any "plasma" zone. The perfusion fluids were made up of frog Ringer to which was added 3 per cent gum acacia, providing a colloid osmotic pressure of 75 mm. water, and varying amounts of standard pituitary extract, horse serum, or both. The experiments show that in these circumstances pituitrine has little effect on the diameter of capillaries and none whatever on their permeability. A web perfused with acacia (3 or 6 per cent) or with acacia and pituitrine will begin to swell in 10-20 minutes and in half an hour the edema will usually be conspicuous. The capillaries become freely permeable to the colloid acacia molecules. By the addition of horse serum this edema can be counteracted and 15 per cent of a stock serum was found to be usually sufficient to maintain the impermeability to colloids—including the acacia—for three hours or more, while 20 per cent was invariably sufficient and 10 per cent definitely insufficient. There is evidently something in the horse serum,

apart from its colloidal properties, which is essential to keep the capillaries of the frog's web in a normal state of permeability. This something is not pituitrine; it does not deteriorate as pituitrine does when the serum is stored in a sterile condition for many months. The power may be inherent in the serum proteins or it may be a special hormone, which it is justifiable to hope may some day be isolated.

It is worthy of note that the capillaries of the frog's skin (of which the web forms part) are normally somewhat permeable to frogs' plasma (p. 310) and give rise to a steady flow of lymph. In Drinker's experiments the drainage of lymph from the foot was carefully prevented. When no swelling occurred for several hours the capillaries must have become less permeable than normally. To my mind this is a suggestive indication of the presence in the serum of a powerful hormone.

#### NOTE

<sup>1</sup> Ito (1926), continuing the work of Isayama on the loss of fluid from the frog's blood, observed when the contractions of the lymph hearts have been brought to a standstill, finds that this loss begins immediately when the lymph hearts are stopped by local application of curari, but after 6 minutes' latency when they are destroyed by cautery. He assumes the existence of a mechanism making the capillaries tight—even to crystalloids—for a short time following injury. While unable to offer any alternative explanation on the basis of the information available I think that the evidence is insufficient to warrant the assumption of such a change in permeability.

## LECTURE XV

### SOME APPLICATIONS OF THE PHYSIOLOGY OF CAPILLARIES TO COMPLEX PROCESSES IN HEALTH AND DISEASE

**I**N the preceding lectures I have brought together and arranged, to the best of my ability, the available information on the qualities and reactions of capillaries. Nobody can realize, I think, more acutely than I do the fragmentary and inadequate character of this information, but I believe, nevertheless, that the attempt should be made to apply this information, such as it is, to a small number of physiological and pathological problems.

Such an attempt will serve as a sort of test. If our information is, on the whole, sound—even if fragmentary—it should not be in serious conflict with what is assumed to be known from other sources, and it ought to serve to throw some fresh light on certain aspects of some of the problems.

Of the problems with which I propose to deal four are physiological: The negative pressure of the thoracic cavity, the absorption of substances from the intestine into the blood, the interaction between the aqueous humor and the blood through the canal of Schlemm, and the formation of glomerular urine; while four are pathological and comprise nothing less than the formidable problems of urticaria, inflammation, circulatory shock, and edema. The physiological problems have been selected partly on account of the peculiar difficulties which they present.

*The "negative" pressure in the thoracic cavity.*

As I have stated in a preceding lecture, the tissue pressure in man and terrestrial mammals is almost everywhere practically equal to the atmospheric. The only notable exceptions to this rule are the abdominal cavity, in the lower part of which the weight of the intestines creates a positive pressure, and the pleural cavity in which the pressure is "negative," that is, on an average some 6 mm. mercury (80 mm. water) below the atmospheric. Several hypotheses have been put forward to explain the mechanism of this negative pressure (Cf. Neergaard, 1927), which is in reality quite simple and can be deduced as a consequence of the normal relations between the capillary blood pressure and the effective osmotic pressure of the blood combined with the anatomical peculiarity of the pleural cavity, viz., that its walls are sufficiently resistant mechanically to allow the setting up of definite pressure differences. As we have seen in the preceding lectures the colloid osmotic pressure of human blood is normally about 360-400 mm. water. The capillary pressure in the walls of the pleural cavity and the surface of the lungs is not known, but we have no reason to believe that it will exceed a few cm. of water in any of the surfaces concerned. There is, therefore, when the capillaries are impermeable to colloids a large excess of effective osmotic pressure which will draw water (with crystalloids) from the cavity into the blood and thereby expand the lungs of the newborn and keep them expanded thenceforward. The force necessary to fully expand the lungs will determine the final pressure reached.

There must be a slow but steady filtration of fluid from surrounding tissues into the pleural cavities, but this fluid is being continuously taken up by the blood.

*The absorption of dissolved substances from the small intestine into the blood.*

Through the intestinal epithelium of an average man there pass per day something like 400 grams of sugar and 100 grams of amino acids. This quantity is dissolved in an amount of water which is not very exactly known but is usually estimated at about 5 liters, making up, therefore, a 10 per cent solution of sugars and amino acids. The solution contains also salts, and to judge from the salt content of the various digestive juices, which is, on an average, somewhat lower than that of the blood, and the amount of salts usually taken with the food, the absorbed solution probably possesses much the same salt concentration as the blood. In any case, its total osmotic pressure is greatly in excess of that of the blood.

With the problem of the transport of this solution through the columnar epithelium we are not here concerned. We will deal only with the problem of its fate after it has entered the intestinal villi, and you will find that this problem, which is usually treated in a few lines in the textbooks, presents very serious difficulties and will require renewed investigation.

It is usually stated that the water and dissolved substances taken up in the intestinal villi are transported practically exclusively through the blood, that there is only a slight increase in the flow of intestinal lymph after a meal, and that this lymph does not contain an increased amount of sugar or amino acids—and even that the concentrations of these substances in the chyle are lower than in the portal blood. If these statements are correct they will, as far as I am able to see, amount to an assertion of secretory qualities on the part of the capillary endothelium in the villi, qualities which it is, as we have seen, unneces-



sary to assume for the ordinary capillaries of the body.

When discussing the lymph flow from the intestine we were led to conclude that the endothelium of the intestinal capillaries is, like the capillary endothelium in the rest of the body, simply permeable to water and crystalloids, and further—in this particular case—to a certain fraction of the blood proteins. If we try to picture on this basis what will happen during absorption when a comparatively strong solution of sugars, amino acids, and salts enters the pericapillary spaces from the epithelium, we cannot but conclude that the osmotically active substances of comparatively low diffusibility must draw water out from the capillaries. At the same time the diffusible substances will, of course, enter the blood stream through the capillary wall, and since the capillary surface is, as shown in the second lecture, very large and exceptionally permeable, while the blood in the capillaries is constantly and rapidly renewed, it is conceivable that a complete equilibrium may be reached, making the percentage concentration of the diffusible substances the same in the chyle as in a corresponding sample of portal blood.

When a complete equilibrium has been reached between the crystalloids on both sides of the endothelium, it is conceivable further that part of the water drawn out from the blood by the initially concentrated solution may be reabsorbed into the capillaries in consequence of the difference in colloid osmotic pressure between the blood and the chyle; but it must be emphasized that such osmotic reabsorption can take place only after complete equalization of the crystalloid concentration, since a difference of less than 0.01 per cent sugar would be ample to counteract the possible excess of colloid osmotic pressure in the blood, and further, that the reabsorption stipulated must, in any case, re-

main incomplete, in view of the fact that the normal relations between the hydrostatic and osmotic forces in the villi lead to a regular transudation of lymph. Reabsorption of water is conceivable only up to the point where the chyle has reached the normal protein concentration of the lymph during fasting.

We come, therefore, to the conclusion that, if the distribution of the absorbed water and crystalloids between the blood and the chyle is to be at all explicable as the result of simple osmotic processes, the concentration of each substance in the chyle must never be lower than in the blood, while the quantity of chyle flowing from the intestine per unit time must be at least somewhat higher than the corresponding quantity of lymph flowing from the empty gut. This conclusion does not agree with the facts as usually stated, but there is, I think, some reason to believe that some of the observations made are incorrect.

Hendrix and Sweet (1917) have analyzed simultaneous samples of blood and chyle during absorption of amino acids and glucose. They found invariably that the amount of amino-nitrogen in the chyle rose considerably and became much higher than in simultaneous samples of blood from the general circulation. The same was the case with the sugar, and in one—but only one—experiment they have compared sugar percentages of the chyle with those of samples from the portal blood and found them to be practically identical.

In some experiments, in which he injected large amounts of 0.3 per cent saline into the small intestine of fasting dogs, Heidenhain (1888) observed a considerable increase in the flow of intestinal lymph, the quantity of injected fluid transported through the lymph channels amounting on an average to about 1/10 of the quantity taken up by the blood, but in other experiments the flow of lymph was scarcely increased.

Heidenhain appears to have thought that the intestinal villi might be so far contracted during absorption that the intercapillary spaces would be nearly obliterated and that almost all the fluid given off by the epithelial cells must enter the capillaries directly. This conception would account for the taking up of practically all the absorbed fluid into the blood, but it does not seem very probable.

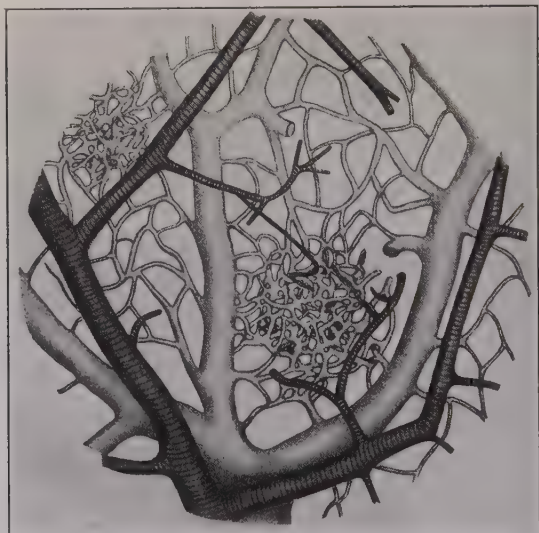


Fig. 90. Nests of small veins in intestinal submucosa of dog. After Mall. Highly magnified.

In his description of the blood vessels of the intestine, Mall (1887) mentions a very curious arrangement of small veins found in the submucosa. In injected specimens these veins appear to the naked eye as "small colored points" present in very large numbers. When "highly magnified" they appear as shown in Fig. 90. Unfortunately the magnification is not stated.

A large number of small veins branching off from the larger vessels coalesce to form a globoid or lenticular "rete." According to the description given by Mall of the lymph vessels of the intestine there can be no doubt that these veins are in close contact with lymph vessels coming from the villi, and it is probable, therefore, that a further interchange of substances may take place, beyond that possible in the villi themselves. It is impossible, however, to form any idea of the quantitative importance of such interchange until the number of these structures has been counted and their surface at least approximately estimated and compared with the capillary surface available in the villi.

Everything considered, I think it most likely that the distribution of the substances absorbed from the intestine will turn out to be explicable on the basis of diffusion, but new experiments are urgently needed.

*The filtration of aqueous humor into the canal of Schlemm and the episcleral veins.*

It has been shown by Leber (1903) and recently confirmed in a series of researches by Seidel (1921, 1922) that the aqueous humor is constantly filtered off from the anterior chamber of the eye through the canal of Schlemm, or ciliary plexus, into the episcleral veins. The canal of Schlemm is generally a circular plexus of small veins firmly imbedded in scleral tissue and separated from the anterior chamber by a layer of endothelium and the fine clefts known as the spaces of Fontana.

According to the somewhat imperfect data given by Leber, the filtering surface of the canal can be estimated at something between 10 and 50 mm.<sup>2</sup>—say 30 mm.<sup>2</sup>

The hydrostatic pressure in the eye is about 25

mm. Hg and the quantity of aqueous humor filtering through is estimated at 6 mm.<sup>3</sup> per minute. When we figure out according to these data the filtering capacity of the canal as defined in the appendix (p. 384), we find 60 cc., or about ten times as much as the filtering capacity of the most permeable collodion membrane made. This agrees well with the fact that the protein-rich aqueous humor produced after puncture of the anterior chamber is easily filtered off and with the observation repeatedly made that Indian ink parti-

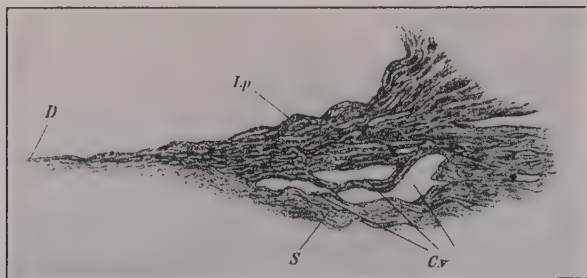


Fig. 91. Section through the border between cornea and sclera *S*. *Cv*. Canal of Schlemm. *Lp*. Spaces of Fontana. *D*. End of membrane of Descemet. After Leber.

cles, the size of which is just submicroscopical, can also pass easily from the aqueous humor into the canal of Schlemm and the episcleral veins, but it raises a serious difficulty.

If the endothelium of the canal is permeable to protein and even to Indian ink particles, a diffusion should take place in the opposite direction, and the aqueous humor ought normally to contain protein. The only explanation which I am able to suggest is that the endothelial cells themselves are impermeable, but that there are a number of extremely fine intercellular passages through which there is a current of filtration



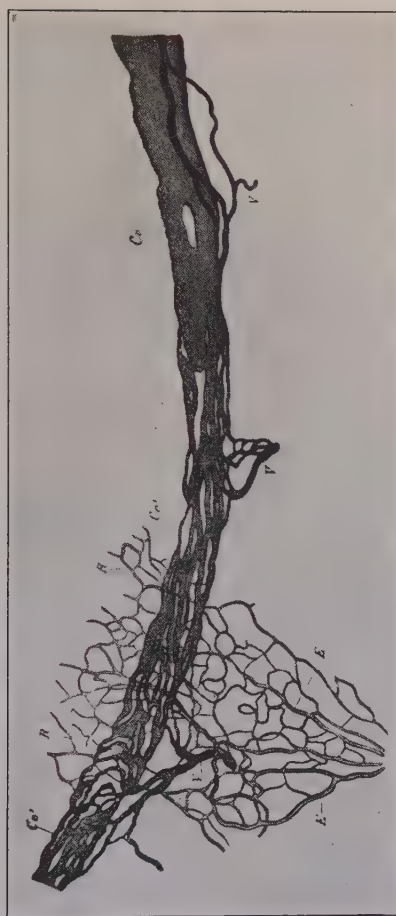


Fig. 92. Part of the canal of Schlemm. *Cv*. *V*. Connections with ciliary veins. After Leber.

which is sufficiently rapid to prevent the entrance of plasma from the blood. Seidel (1927) has recently shown that only the smallest Indian ink particles will pass through at first while the passage is blocked a few minutes later by the larger particles.

The canal of Schlemm in man is not a part of the regular venous system, in so far as no capillaries open into it, but is a sort of diverticulum opening through numerous small passages into the ciliary veins. Seidel (1922) is of opinion that the canal does not normally contain blood but is filled with the fluid filtering off from the anterior chamber.

#### *Glomerular filtration.*

According to modern conceptions urine is formed by a process of filtration in the glomeruli of the kidneys, the capillaries of which are taken to be permeable to all crystalloids and impermeable to colloid proteins. The filtrate is modified by selective reabsorption in the tubules of certain constituents (e.g., glucose) and water and further modified by diffusion of part of the dissolved substances from the concentrated urine back into the blood (Rehberg, 1926). Rehberg has found one substance, creatinine, a normal constituent of the filtrate which is not reabsorbed and which can be assumed not to diffuse back to any appreciable extent. When, therefore, the concentration of creatinine in a sample of urine is compared with the corresponding concentration in the plasma or in an ultra-filtrate from blood the ratio between the two concentrations gives an indication of the quantity of water reabsorbed and makes it possible to calculate the initial amount of filtrate. From his determinations Rehberg concludes that the two million glomeruli present in normal human kidneys (Vimtrup) will produce a maximum of 200 cc. filtrate per minute of which at least 180 cc. and even up to 199 cc.

become reabsorbed during the passage through the tubules. This enormous rate of filtration and reabsorption is considered by several authors as a serious difficulty which renders them skeptical toward the whole filtration-reabsorption conception.

By combining the estimates of glomerular surfaces arrived at by Vimtrup with the determinations of capillary filtration given by Landis it is possible to obtain a kind of test, though it should be remembered, of course, that all the figures used are rather rough approximations, while it is quite possible that the filtering capacity of human glomerular capillaries differs considerably from that found for the mesenteric vessels of the frog.

From Landis' figures a filtering capacity of 3.4 cc. per minute per 100 sq. cm. under a pressure of 1 atmosphere is deduced, and special experiments detailed above (p. 325) show that this can be increased to 9.1 before the capillaries become permeable to any fraction of the protein. Vimtrup gives the total glomerular surface of both human kidneys as 15,000 sq. cm. The available filtration pressure is the blood pressure in the glomeruli minus the colloid osmotic pressure of the blood. It can be deduced from anatomical considerations which are to some extent reinforced by physiological experiments (Starling, 1924) that the glomerular blood pressure is very high, but just how high we have no means of ascertaining. I take the filtration pressure to be at least  $1/20$  atm. ( $= 500$  mm. of water) and probably not above  $1/10$  atm. ( $1,000$  mm.). Assuming the glomerular capillaries to have the same permeability as the frog's normal mesenteric capillaries we could get a filtration per minute of 25 to 50 cc. while assuming the permeabilities observed just before protein begins to pass through we should have filtration rates of 65 to 130 cc. These figures are suffi-

ciently close to Rehberg's estimate of the actual filtration to serve as a support to the general theory.

The experiments of Starr (1925, 1926) indicate that the glomerular capillaries in man and mammals like those of the frog's mesentery are very sensitive to oxygen lack which makes them permeable to protein.

In the following pathological conditions capillary reactions play an important and more or less conspicuous part: Urticaria, inflammation, circulatory shock, and edema. I do not mean to convey any systematic idea by this enumeration, which only represents the order in which it is convenient from my point of view to discuss them.

#### *Urticaria and inflammation.*

The symptoms used of old to characterize inflammation, viz., rubor, calor, turgor, and dolor, correspond very closely to the "triple response" of Thomas Lewis. The redness is brought about by opening up and dilatation of capillaries and venules, the heat by the increased flow of blood due to dilatation of arterioles, the tension by exudation of fluid from the vessels due to their increased permeability, and the pain finally by the stimulation of nerves, causing in the skin also the surrounding flare. We have learned from the researches of Lewis that this symptom complex is normally due to liberation of H-substance, a product of tissue cells when exposed to injury of whatever kind, and with Lewis we take the whole reaction to represent an essentially defensive mechanism counteracting the injury without calling upon the central organization of the body.

The description given and expressed in the terms either of the "triple" response or of the four cardinal

symptoms fits admirably to all those cutaneous reactions which can be classed as "urticarial," but when we apply it to inflammation, properly so called, we miss something essential. While admitting that the word inflammation is often used in a very comprehensive sense and to cover processes (e.g., of cell proliferation) which are probably only indirectly if at all related to those here considered, I believe there will be a general agreement that the emigration of leucocytes should be included as perhaps the central reaction in inflammation. Emigration of leucocytes is brought about by substances having a positive chemotactic action, and it is known that there are several such substances the action of which can be demonstrated *in vitro*. It is very significant that histamine, the prototype of Lewis' H-substance, has been shown by Miss Wolf (1921) to attract the leucocytes of man and the dog in concentrations down to 0.000025 per cent. Although this action was missed *in vivo* by Bloom (1923) there can be little doubt that it exists (Wolf, 1923) and in so far as histamine is truly representative of the H-substances we have to class the urticarias with the true inflammations. Török (1928) gives a number of instances in which the emigration is demonstrated in experimental and spontaneous urticaria, and one of these obtained with the poison of *Urtica urens* is here reproduced (Fig. 93). Nevertheless, it cannot be denied that emigration is not a very conspicuous symptom in urticaria, and the point I wish to make is that we probably have in most inflammations besides the H-substance or substances further chemotactic agents which may be substances directly introduced (e.g., cantharidine); products of bacterial metabolism or substances arising from abnormal cell metabolism or disintegration as in the inflammation caused by abrine (cf., p. 222).



The main symptom in urticaria is the wheal brought about by a considerable increase in the permeability of the minute vessels of the skin and a consequent filtration of a fluid, the composition of which comes very close to the blood plasma. A wheal is normally formed in the papillary layer of the cutis and along the first venous plexus. It spreads horizontally, maintaining a sharply defined limit and produces a hard infiltration

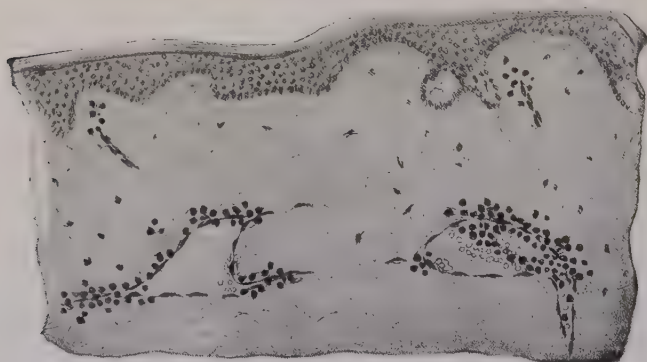


Fig. 93. Section through wheal produced by nettle poison.  
Emigration of leucocytes. After Török.

and swelling of a thin layer of tissue. A similar infiltration can be produced by intradermal injection of saline. Both the artificial and the natural wheal disappear very slowly from the normal skin. The natural wheal fluid cannot become absorbed by the blood vessels on account of its protein content, and the absorption of injected saline is practically prevented by the pressure, which renders the tissue nearly bloodless. Whether the channels connecting the papillary layer with the subcutis are also blocked by pressure or whether they are normally too feebly developed to drain off the fluid cannot be decided on the available

information, but when, at some later stage, the fluid finds its way through the skin the passages remain abnormally open for some time, and a fresh wheal cannot be formed even by a rapid filtration of fluid. Exudation in the deeper layers of the cutis does not produce a sharply delimited wheal (Török, 1928) and the opportunities for draining off are evidently better. For some reason, so far obscure, the exuding plasma sometimes finds its way into the germinative layer of the epidermis and forms a blister, the content of which seems usually to become entirely shut off from the lymph channels, perhaps by the keratinization of the cells forming its lower border.

The vascular reactions in urticaria and inflammation are on the whole independent of the nervous system and run essentially the same course in denervated tissue as where the sympathetic and pain innervation is normal (Lewis, pp. 72, 118; Török, 1928, p. 90). The "reflex hyperemia" cannot appear, of course, after degeneration of the pain fibers, but this does not affect the local process. In tissue deprived of its sympathetic innervation the reaction may be somewhat modified; dilatation of capillaries and increased blood supply may begin earlier and cases are on record (Dreyer and Jansen, 1905) where recovery is accelerated while in others it is hindered and delayed.

In the main, I believe, we must agree with Ribbert (1909) in considering inflammation as a complex protective and restorative reaction in which the vessels and the elements of the blood take their part along with the other tissue elements. I would emphasize the point, however, that though the reaction in its entirety is certainly beneficial to the organism, some of the partial reactions, at least of the vascular system, are often noxious. The development of complete stasis, for instance, in a large number of capillaries cannot but

expose the tissues to grave dangers. It is, to say the least, difficult to see how the edematous swelling of inflamed tissue can be beneficial.

It may very well be worth while to study the vascular reactions during inflammation with the object in view of getting them under control, of restraining them at the points where they become harmful, and of helping them on where they are beneficial. The calcium therapy inaugurated by Chiari and Januschke is, if rightly interpreted, to be considered as an attempt in this direction. The experiments of Poulsson (1926) show that pituitrine in suitable doses is able to diminish exudation in experimental cases of inflammation and edema.

#### *Circulatory shock.*

The word shock has been used, and is often used, to denote very different pathological conditions, some of which have, perhaps, nothing whatever in common beyond the symptoms of collapse. Here we have to deal only with circulatory failure of the type described in the ninth lecture as resulting in certain animals from a large dose of histamine and there spoken of as histamine shock. You will remember that the analysis made by Dale and Laidlaw showed that the symptoms are due to a general capillary dilatation which takes up so much of the available blood that the return flow to the heart fails and the arterial blood pressure becomes gradually very low.

A number of researches, among which should be especially mentioned that splendid example of what can be achieved by hearty coöperation, the Reports on Wound Shock and Hemorrhage by the Special Investigations Committee on Surgical Shock (Medical Research Committee, 1919), have shown that a condition essentially similar to histamine shock can be brought

about by severe traumatic injuries, and the work of the committee has proved conclusively that traumatic shock is due primarily to the action of toxic substances formed without the intervention of microorganisms in the injured tissue and distributed throughout the body by the circulating blood itself. It is evident now that these substances must belong to the class of H-substances.

A very essential feature in the etiology of shock is the vicious circle which is set up by the poisoning of the capillaries. When the circulation begins to fail the blood supply to the tissues suffers and this in turn leads, by reason of oxygen lack or by reason of the diminished supply of tonic hormone, to still further dilatation and increase in permeability. At more advanced stages the permeability of the capillary wall is so far increased that loss of plasma occurs, thus aggravating once more the failure of the circulation.<sup>1</sup>

Further important results of the committee's investigations are the demonstrations that anesthetization with ether and exposure to cold are apt to aggravate severely the conditions of shock. The effects of anesthetics have been discussed in Lecture IX and are easily understood, but the effects of cooling appear to be more complicated and a perfectly satisfactory explanation has not been found so far. The degrees of cold likely to occur should, in ordinary circumstances, give rise rather to capillary contraction, at least in the skin, but it is quite possible that the capillaries in the internal organs react differently when the body temperature becomes actually lowered. This point will require further investigation.

States of circulatory failure, similar in mechanism to traumatic shock in so far as they are mainly of toxemic origin, are, I believe, not at all infrequent. I am very imperfectly acquainted with the anaphylactic

shock, the mechanism of which is perhaps very complex, but which appears, according to Biedl and Kraus (1909), to have important features in common with traumatic shock; but I would draw attention to the symptoms in severe peritonitis and to those which often develop in patients after more or less extensive burns.

H. Olivecrona (1922) has shown very clearly that experimental peritonitis in rabbits, cats, and dogs leads to typical circulatory shock showing the rapid pulse, the low blood pressure, the cyanotic pallor, and the characteristic reduction in the volume of circulating blood. Evidence is presented to show that this form of shock is due to poisons, probably disintegration products of proteins, liberated into the blood.

It is well known that after burns, especially after more or less extensive scalding, a state of collapse, ending in the patient's death, may develop in one to two days in cases where the primary lesions are comparatively slight and do not involve any organ of vital importance. These cases, for which bacterial infection could not possibly be made responsible, have, of course, aroused considerable interest and have been studied extensively, but so far as I am aware (I am not sufficiently familiar with clinical literature to feel sure), the only definite conclusion reached is that the symptoms are due to intoxication. When, however, these symptoms are studied in the light of the information obtained about circulatory shock, the similarity with the shock symptoms is, I think, very striking.

There is the typical fall of blood pressure, the rapid and very weak pulse, the concentration of the blood, as shown by the increase in the corpuscle count. It has been noted (Helsted, 1905) that the intoxication from burns often leads to an increase in body temperature, a point in which there is apparently a difference between



the toxic products from traumatized and from burnt tissue.

Cevario (1921) has made experiments on rats joined in pairs by lateral celiotomy. One of each pair underwent experimental scalding of a part of the skin. Both animals suffered to the same extent, which shows that toxic substances circulating in the blood must be responsible for the symptoms.

Edmunds and Johnston (1928) have recently observed that in shock brought about experimentally by diphtheric toxin a pronounced rise in blood pressure can be obtained by pituitrine injections while adrenaline is without effect.

#### *The formation and absorption of edema.*

The general problem as to the causes of edema is one of extreme complexity, and in order to reach any valid conclusions we must establish certain distinctions between those types of edema which are more or less dependent upon the state of capillaries and their pressure relations and those which are not.

To these latter types all those cases of edema should be referred in which the edema is intracellular—is brought about by a swelling of the tissue elements themselves, while the intercellular, pericapillary, and lymph spaces do not contain more than the usual amount of fluid.

A very instructive case of intracellular edema has been described by F. Mendel (1922). It was characterized by a swelling of the cutis cells, which was present over the whole body but especially pronounced in the legs. It had brought about an increase in the weight of the patient from 77 to 95 kg. There were no symptoms on the part of the kidneys or the heart. When the patient was deprived of sodium chloride in his food a rapid excretion of water began in a few days,

and in a week the weight was reduced to normal. An experimental return to a diet containing salt brought back the edematous symptoms.

Mendel points out that, in this case, we have to do with an abnormal affinity of the cutis cells for NaCl. They take up this substance from the blood so long as it is present in excess, or even in normal amount, in the blood; and, for obvious osmotic reasons, they must take up a corresponding amount of water. The state of the capillaries has nothing whatever to do with the process. They can neither hinder nor accelerate the absorption of salt and water, both of which substances diffuse freely through the endothelium, whether it is normally or abnormally permeable.

Without expressing any opinion regarding the relative importance from a clinical point of view of intracellular and intercellular edema, respectively, I would only emphasize the fact that from the point of view of capillary physiology and pathology the cases of intracellular edema should be ruled out. They belong to an altogether different category.

The cases of intercellular edema with which we have to deal are themselves sufficiently complicated and are controlled by so many factors that great caution must be exercised in drawing conclusions regarding them. The exudation and eventual reabsorption of fluid in the intercellular spaces will depend upon the capillary blood pressure, the colloid osmotic pressure of the blood, the permeability of the capillary wall, the efficiency of the lymph flow, and the metabolic activities of the tissue cells. It cannot be surprising that the process resulting from the interaction of these factors is often difficult and sometimes impossible to disentangle.

If we rule out to begin with the possible changes in capillary permeability and in cellular activity, we can

put down as the simplest type of intercellular edema the filtration edema which will be produced in any tissue whenever the filtration pressure—the capillary blood pressure—exceeds the effective osmotic pressure of the blood. So long as the excess is slight the transudate can probably be removed through the lymph channels as rapidly as it is formed and no visible edema results, but the rates at which removal can take place in different tissues have not been determined. A filtration edema can be brought about either by a sufficient decrease in the colloid osmotic pressure of the blood (a hydremic plethora) or by an increase in the capillary pressure or by both factors acting in the same direction.

Examples of experimental filtration edema have been mentioned in the preceding lectures. It is easily produced by the hydrostatic venous pressure in the human feet when these are allowed to hang down. In an interesting series of experiments Mende (1919) has measured the rate and degree of swelling in the arms of normal persons after the application of a rubber cuff in which a definite pressure was maintained. When the pressure was raised to 100 cm. water, an immediate increase in volume of about 40 cc. was observed, due, of course, to the filling up of the vessels, especially the veins, with blood. During the next fifteen minutes a further increase of about 60 cc. took place, which can be shown to be due partly to dilatation of capillaries and venules, partly to the formation of a filtration edema. This increase was continued, though more slowly, for a further period of nearly twenty minutes, when decompression took place after a total increase in volume of 120 cc. On decompression the volume went down about 70 cc. in a couple of minutes and thereafter took thirty-five minutes to regain the original volume. Mende found that, by the application of 63 cm.

water pressure for twenty-two hours, he could produce a definite edema which would disappear in two to four hours after decompression, while with 50 cm. he obtained only passive hyperemia which would go back immediately on decompression.

If the pressure applied could be taken to indicate the venous pressure reached in Mende's experiments they would furnish an indication of the effective osmotic pressure in the blood of his patients. Mende shows, however, that in his experiments this is not the case, owing to peculiarities of the apparatus employed, and all we can say is that the venous pressure was certainly considerably lower, and probably some 20 cm. of water lower, indicating an effective osmotic pressure of something between 30 and 40 cm. You may remember that in osmometers just impermeable to protein we found an average osmotic pressure of human blood amounting to 46 cm., with individual variations from 40 to 51 cm. The lower pressure to be deduced from Mende's experiments is probably due to an increase in capillary permeability brought about by the dilatation. The comparatively slow disappearance of the edema after 63 cm. pressure points to the probability that the fluid contained some protein.<sup>2</sup>

A simple filtration edema comparable to that produced experimentally by Mende and others is of frequent occurrence although generally of slight extent in normal subjects. The increased size of the feet often noticed in the evening is a case in point. Thompson, Thompson, and Dayley recently (1928) studied the changes in composition of the blood taking place in subjects when standing still for 20-30 minutes. They observe increases in red cell count and volume indicating concentration of the blood and corresponding increases in the protein content of the plasma showing that the fluid lost is, at least approximately, protein-

free. The loss of water from the blood amounted on an average to 290 cc. with individual variations from 190 to 475 cc. There can be no doubt that this fluid has filtered out through the capillaries of the lower extremities.

Vollmer and Lee (1927) describe a similar concentration of the blood taking place in babies when crying violently. In some cases the face becomes definitely edematous. I suppose the mechanism to be an inhibition of the venous return to the thoracic cavity producing a sufficient increase in the general venous pressure to cause filtration, but the point ought to be studied directly.

In cases of cardiac insufficiency the venous pressure is increased and according to Eyster (1926) pressures of 250 mm. of water in the central veins are not uncommon. Corresponding increases in the capillary pressure in the organs are to be inferred, but direct determinations are lacking. Iversen and Nakazawa (1927) have shown that when edema develops in bed-ridden cardiac patients we find, in addition to the supposedly increased capillary pressure, colloid osmotic pressures of the blood definitely lower than the normal, and it seems that a combination of both factors is generally necessary to bring about edema. They observed in several cases an absorption of the edema when the colloid osmotic pressure rose so as to approach the normal value. Edema was present in all cases when the colloid osmotic pressure was below 230 mm. of water and usually when it was below 300, while in persons with an efficient circulation the osmotic pressure of the colloids can be considerably reduced without any edema resulting. Iversen and Nakazawa find that the reduction in effective osmotic pressure in their cardiac patients is due to albuminuria through which the proteins are constantly lost, and they make



it probable that the albuminuria is a consequence of the inefficient circulation through the kidney and due primarily to the failure of the heart.

Many cases of ascites in which the fluid in the abdominal cavity shows a low protein content are due to a cirrhosis of the liver increasing the resistance until the pressure in the portal system exceeds the colloid osmotic pressure of the blood. Iversen (1928) proposes as a treatment for severe cases the reduction of the blood flow through the portal system by removal of the spleen and part of the intestine.

In the cases so far considered, which could be supplemented by further examples, the primary cause of the transudation of fluid is an abnormally high hydrostatic pressure in the capillaries while the effective osmotic pressure of the blood is either normal or secondarily diminished. There are other cases, however, in which a reduction of the colloid osmotic pressure is the primary factor. The first case of this type was studied by Hagedorn, Rasmussen, and Rehberg in my laboratory during my absence as Silliman lecturer (October, 1922) and briefly reported in a note to the first edition. The percentage of protein in the patient's blood was somewhat reduced (to 5 per cent), but the osmotic quality of the protein was very inferior, the pressure measured being only about 100 mm. of water. Filtration of fluid from the capillaries to the tissue spaces was, therefore, unavoidable in many parts of the body. The disease was diagnosed and a clue to the situation was found by a study of the urine which contained 2.8 per cent protein with an osmotic pressure of 240 mm. The glomeruli were, therefore, filtering off the smallest and osmotically most active protein molecules.

A number of similar cases have since come to light (Gowaertz, 1924; Schade und Claussen, 1924). I repro-

duce a small table from the paper by Iversen and Nakazawa (1927) showing cases of different severity.

Diagnosis	Blood			Urine			Edema
	Protein per cent	Osm. press. mm. H <sub>2</sub> O	O.p. ÷ % protein mm. H <sub>2</sub> O	Protein per cent	Osm. press. mm. H <sub>2</sub> O	O.p. ÷ % protein mm. H <sub>2</sub> O	
Nephrosis	6.77	89	13	1.65	185	112	+++
Amyloidosis	3.94	80	20	1.0	76	76	++
Mercurial nephrosis	4.81	175	26	0.9	85	96	++
Chronic nephritis	7.2	292	41	0.5	50	100	—
Acute nephritis	6.12	239	39	0.6	64	106	+
Acute nephritis	6.36	270	43	0.125	?	?	—

In this group of cases edema is absent when the effective osmotic pressure is above 250 mm. The primary cause is the albuminuria, and it is significant that there is usually a certain relation between the protein concentration of urines and the specific osmotic pressure of their protein which is the higher the lower the concentration, while, as you will remember, the reverse is the case when a protein solution is simply diluted. Iversen and Nakazawa give a table to illustrate this relation from which I have constructed Fig. 94. The urinary protein shows in all cases a higher activity than that of normal blood while at the lowest concentrations the activity becomes very high. The glomeruli evidently constitute an effective ultra-filter which can become permeable to and separate off the most active protein fraction. Since the protein in the glomerular filtrate is concentrated between 50 and 200 times during the passage through the tubules, we have to assume that these highly active molecules are present in the blood in very low concentration only.

The edema fluid in the cases of filtration edema through normal capillaries now described is never really protein-free. Usually, however, it contains less than 0.1 per cent, but the percentage may be higher, especially in ascites fluid. The origin of this protein has not been determined. It may be the tissue cells, but the possibility cannot be excluded that in some of the cases studied a number of capillaries have not re-

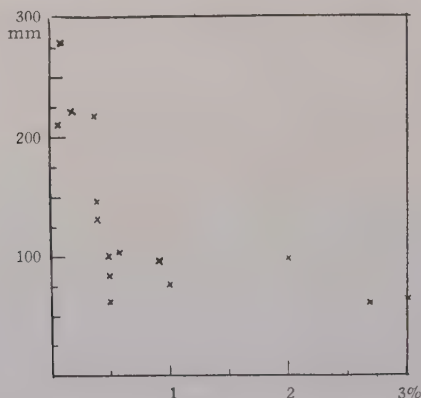


Fig. 94. The relation between the protein concentration of urines and the specific osmotic pressure of the protein. Abscissa, protein in urine. Ordinates, pressure per per cent protein.

mained normal but have become permeable to protein. In practice we cannot, therefore, distinguish so sharply as would be desirable theoretically between the cases of simple filtration edema and that other group in which the filtration is due to an increased permeability of the capillary wall due to damage of some kind, while the capillary blood pressure and the total colloid osmotic pressure of the blood may remain unchanged.

It is worthy of note that with the exception of the glomerular capillaries and the mesenteric capillaries

of frogs, according to Landis' experiment referred to above (p. 322), we have no evidence of any selective permeability on the part of injured capillaries. In those cases, admittedly too few, in which the colloid osmotic properties of edema fluids have been studied, the pressure calculated for each per cent of protein has been found to be the same as that of blood diluted to the same protein concentration or lower (Iversen, 1928).

While the protein-free fluid filtered out through normal capillaries can and will be reabsorbed directly into the blood when the colloid osmotic pressure of this fluid is raised above its hydrostatic pressure in the capillaries, a fluid containing protein cannot possibly be completely reabsorbed.

So long as the increased capillary permeability persists the transudation will go on, and when the capillaries return to normal they become impermeable to the protein in the transudate, the osmotic pressure of which will prevent also the reabsorption of the water and crystalloids.

For the absorption of edema fluids containing protein two different mechanisms are at present recognizable. One is the return to the blood by way of the lymphatics, and the experiments of J. H. Lewis (1921), referred to in the twelfth lecture, show conclusively that protein is transported along this route. The other has been pointed out in a paper by Landsberg (1921), who observed that the protein in a pleuritic exudate was gradually broken down by enzymes, produced, no doubt, in adjoining cells. The cleavage products would be able to diffuse into the blood and a direct reabsorption of the edema thus becomes possible.

Such a mechanism seems to be especially appropriate for the pleural cavity from which the lymphatics are probably unable to take up fluid owing to the nega-

tive pressure. Fluid contained in the abdominal cavity and even suspensions of blood corpuscles and other particles will readily enter the lymphatics in the lower surface of the diaphragm (Siperstein and Sansby, 1923) and Florey has recently shown (1927) that this passage takes place through preformed openings while its driving force is the positive pressure in the abdominal cavity.

In the clinical literature numerous cases are recorded, of which characteristic examples are given by Volhard (pp. 1163, 1255, 1274), showing rapid absorption of edema fluids after diuretic drugs—caffeine, theobromine, calomel, and others. The mechanism of these cures is at present unknown, and I would venture to remark that it appears idle to speculate upon it until the facts of the cases have been more completely ascertained.

Ever since the splendid work of Claude Bernard (1852) inaugurated the study of vasomotor mechanisms, that branch of physiological science which deals with the regulation of the circulation has been a favorite subject of research and has exercised a profound influence upon physiological and pathological thought generally.

Until recently the word vasomotor was used as synonymous with *arteriomotor*. We have realized now that beyond the well-known arteriomotor we have certain *capillariomotor* mechanisms and, though the systematic study of the capillaries and their reactions is still in its infancy, it has shown a vigorous growth both as a branch of pure science and with regard to its direct and indirect applications for the benefit of mankind, and it is safe to predict that it will be recognized



ultimately as of equal importance with the study of the heart and the arterial system.

It is my hope that these lectures may help in some measure to promote the growth of this young branch of physiology and to arouse an active interest in a study which, by revealing more and more the beautiful correlation of activities in the organism, will further the purpose for which the Silliman Memorial Lectures were established.

### NOTES

<sup>1</sup> According to a proposal originally made by Bayliss (1916), after consideration of the importance of maintaining the colloid osmotic pressure of the circulating fluids, circulatory shock is now very generally treated by intravenous injection either of blood or of an isotonic solution of gum acacia.

Injections of isotonic and hypertonic solutions of various crystalloids have been exhaustively tried, but found to be useless, and it is easy to see that in cases where the permeability of the capillary wall is increased such solutions must leave the circulation very rapidly.

According to experiments made in my laboratory the effective osmotic pressure of gum depends very largely upon the salts of the solution, being very high in pure water, as shown previously by Gasser, Erlanger, and Meek (1919). When dissolved in normal saline, a solution of 6 per cent gum acacia has nearly the same colloid osmotic pressure as human blood, while its viscosity is slightly higher, and, when pure, it appears to be a perfectly innocuous substance. We have found the diffusibility of gum through collodion membranes to correspond very closely to the least diffusible fraction of the blood proteins, so that it will probably be held back in capillaries which have become permeable to the greater part of the normal plasma colloids.

The commercial gum contains salts, and it has been stated by Bayliss (Med. Res. Com., 1919) that it is sufficient to dissolve the gum in 0.9 per cent sodium chloride, since the gum contains sufficient calcium and potassium salts.

Zondek (1921) has attempted to explain the effects of gum solutions as due to their calcium content, which he assumes to be very high. Determinations made by Mr. Rasmussen in my laboratory show, however, that the effective concentration of both calcium and potassium in a 6 per cent solution is only about double the concentration of the same substances in Ringer, that is, the concentration of diffusible Ca and K ions correspond to 0.06 per cent  $\text{CaCl}_2$  and 0.05 per cent  $\text{KCl}$ . The gum should, therefore, make an almost ideal provisional substitute for blood plasma. Bad re-

sults from gum have been reported, but are apparently due to the use of inferior preparations. Bricker and his collaborators (1926) have used gum with good results on human cases and Ueki (1924) has made extensive tests on the Langendorff heart preparation and on cats bled to various extents down to 30 per cent of the blood volume. Ueki recommends the use of gum purified by an electro-osmotic process.

It should be clearly understood that in cases of shock the gum or blood injected can act only by relieving the circulatory failure and breaking the vicious circle. If the capillaries go on dilating under the influence of the poisons formed, the relief can be only temporary, and if the shock has reached such a stage that the capillaries have become permeable to gum, the injection will prove useless.

<sup>2</sup> Similar series of experiments were made recently on the human leg by Drury and Jones (1927) using an ingenious water plethysmograph to measure the rate of edema formation. Unfortunately the absolute relation between capillary pressure and rate of filtration and the pressure necessary to produce a just perceptible increase in volume cannot be calculated from their published experiments, because these authors have made the error of not taking into account the counter pressure of the water in their plethysmograph.

APPENDIX  
ON  
METHODS FOR THE STUDY OF CAPILLARY  
ANATOMY AND PHYSIOLOGY

*Observations of capillary circulation.*

THE technique of capillary observation differs considerably according to the kind of animal and method of illumination employed (transmitted or reflected light).

Observations can be most easily made on small cold-blooded animals by transmitted light. The tails of young larvae of newts and frogs are specially suitable, and Vimtrup (1922) describes the following arrangement which will immobilize a tadpole without the use of narcotics which should be avoided as much as possible in observations on normal capillaries.

Two lumps of plasticine are placed on a slide and modeled so as to hold the body of the larva without exerting pressure. The tail to be examined can be placed under a cover glass on plasticine feet or a very thin sheet of mica. The body is covered with a narrow strip of filter paper, the microscope tilted at a suitable angle, and drops of water are arranged to fall on the filter paper once or twice per minute. This arrangement is specially suitable for the study of Rouget cells in the living state.

In the study of grown frogs it is equally important to immobilize the body with as little interference as possible and to keep it moist. The web, tongue, bladder, mesentery, and lungs are suitable organs for ob-

servation by transmitted light. The web is spread by means of triangles cut from object slides and placed between the toes. Of European frogs, only the brown species can be used and even in these the circulation is often obscured by the pigment. Small and light-colored animals are most suitable. The tongue is pinned out on a cork frame surrounding a piece of glass as shown in Fig. 21, p. 66. Hedgehog quills are specially suitable for pinning out. They do not irritate the tissue and can be cut off with scissors so as not to be in the way.

To expose internal organs a small incision is made with scissors or a cautery at a point near the middle line where no large vessels are found. The skin is lifted and the inner surface inspected to avoid the visible vessels in the further cuts.

The bladder is made to project through the opening by filling it with saline from a pipette inserted through the cloaca.

The lung is moderately distended with air through a bent glass tube. It is generally unnecessary to have a permanent cannula in the larynx. A flat surface for observation must be provided by placing a lump of plasticine beside the bladder or lung and pressing a piece of object slide or thick cover glass down upon it.

The mesentery is exposed through a lateral incision which is best made by means of a cautery to avoid loss of blood. The opening is suitably enlarged by scissors and a loop of intestine cautiously drawn out and exposed over a transparent glass stage. It should not be fixed by needles. A constant slow drip of saline keeps the preparation moist and clean.

On warm-blooded animals certain transparent appendages such as the ears of some races of rabbits, rats, and mice (Fröhlich u. Zak, 1924) and the bat's wings (Carrier, 1926) can be utilized for observations by

transmitted light. To observe the really normal circulation in internal organs of mammals is extremely difficult as shown by the experience of Rich (1921). Results of considerable value can be obtained, nevertheless, when caution is exercised. The various arrangements to bring the organs of mammals under the microscope cannot be described here, but reference should be made to a very convenient trough and tray for observations on the mesentery of rats constructed by Florey (1926). The organs must be rigorously maintained at the right temperature. The common practice of irrigating them with Ringer solution is unsatisfactory, but difficult to replace. It is probably of some importance to maintain tensions of carbon dioxide and oxygen that are at least approximately correct—about 5 per cent of an atmosphere for each gas.

The illumination employed for visual observation should not be stronger than necessary, and it is often useful to filter the light to remove the red and violet ends of the spectrum. In cases where the circulation of the red corpuscles is the object of study a filter made up of Flavazin and Naphthol green is often extremely useful. Photographic plates are treated with 1 per cent solution of the two dyes and joined together. The combined filters will let through the light between orange and blue green. The absorption bands of hemoglobin occupy the central part of this region and the contrast between the blood and the tissue is, therefore, greatly enhanced.

*Observations by reflected light* (Lombard, 1912; Weiss, 1916) require a strong source of light. The condensed rays from a small arc lamp are for most purposes superior to any other illumination, provided they are suitably filtered. For low power observations a Greenough microscope and oblique illumination through the contrast filter for hemoglobin is the best.



The application of a cover slip (Schur, 1920) may sometimes prove advantageous. High powers can be used with the vertical illuminator (Vonwiller, 1924) and Crawford and Rosenberger (1926) have introduced the use of polarized light to extinguish the reflection from the surface of the preparation.

In the study of the human skin the surface must be smoothed and the transparency increased by the application of a drop of a highly refractive substance. Oil of paraffin will give good results and glycerin can be used in cases where oil is objectionable. Lewis (p. 172) recommends the use of absolute alcohol to dehydrate the epidermis before the oil (cedar oil) is applied. In other cases he produces a blister, removes the epidermis, and covers with paraffin oil.

#### *Photography.*

In many preparations the capillary circulation can be easily photographed by means of cameras of the "Phoku" type allowing simultaneous observation. The strong light necessary for instantaneous photography should not be kept on longer than necessary. For the purpose of instantaneous photography by reflected light Sheard (1924) has used the device of increasing for a few seconds the current through the illuminating arc lamp. Cinematographic arrangements have been described by Krogh and Rehberg (1924) and Crawford and Rosenberger (1926).

#### *Anatomy of capillary systems.*

The arrangement of capillaries is studied on injected specimens. Many different injection fluids can be used, but from my personal experience I prefer gelatine mixed with fluid Indian ink. The vessels are washed out with Ringer solution, and if this is introduced gradually into a vein of the narcotized animal

the heart can accomplish most of the washing. In striated muscles, in many parts of the intestine and probably in other organs complete injections are difficult to obtain, and procedures that are sure to give such have not been found. It may be useful to leave the organs many hours or even days after death before injecting and to tie off the veins at the end of the injection and force open the capillaries by a high pressure.

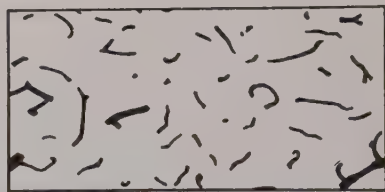
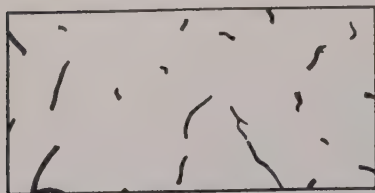
From the injected specimens suitable pieces are cut out and, for quantitative purposes, carefully measured. It may be useful to freeze the specimens and make measurements in the frozen condition. Pieces of tissue like the kidney cut out to present flat rectangular sides are pressed loosely against ordinary writing paper where they will leave impressions which can be measured afterwards. I have always pinned out the pieces of muscle and other organs selected before fixing. The fixing fluid should be chosen so as to cause as little shrinkage as possible, but in any case the dimensions after imbedding must be measured and allowance made for the shrinkage which is as a rule different in different directions.

For the study of the general arrangement of the small vessels thin pieces of tissue or thick sections are cleared so as to become transparent. Diaphanol is sometimes useful for this purpose, but generally the end can be attained by dehydration and treatment with clove oil. These preparations should be examined with the Greenough binocular microscope. For quantitative purposes the technique of cutting and mounting must vary according to the tissue.

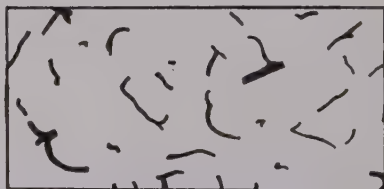
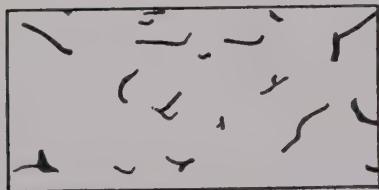
Striated muscles are cut in fairly thin transverse sections after accurate determination of the shrinkage, and countings are made of the dots representing the sections of capillaries. It is generally useless on arti-

ficially injected specimens to measure capillary diameters which have become altered to a variable and uncontrollable degree.

From membranes and other tissues in which the capillary system is arranged approximately in one plane, photographs are taken and suitably enlarged. On these the total length of capillaries in a given area can be determined by running a small measuring wheel along them.



Figs. 95 and 96. Blood vessels in  $20\mu$  sections of hypoglossal and vestibular nuclei of brain of newborn albino rat. After Craigie.



Figs. 97 and 98. Blood vessels in corresponding sections of adult male rat.

To determine capillary lengths in the three dimensional very irregular system of the rat's brain Craigie (1921) made sections of  $20\mu$  thickness, which he found to represent a suitable fraction of the size of capillary meshes and in these sections of which Figs. 95-98 represent specimens he measured the capillary loops

one by one making due allowance for foreshortening in each measurement. It would probably be easier and just as accurate to calculate the average degree of foreshortening and to use a measuring wheel on suitably enlarged photographs.

*Capillary contraction and dilatation.*

The simple fact of capillary contractility is studied by direct observation. To solve the more general problem concerning the state of the capillaries in a certain organ under certain conditions vital injections with subsequent fixation of suitable pieces of tissue constitute the general method.

Several substances can be used for vital injection. In his studies of the omentum Rich made the animal's own blood corpuscles available by a suitable staining (Slonimski, 1927), but according to my experience this simple method will not generally give pictures with sufficient contrast especially for quantitative purposes. Jores (1927) uses trypan blue and this or some other colloidal and non-toxic dye may be suitable in several cases, but the concentration employed must be comparatively high to produce the necessary contrast. Indian ink, which I have formerly used, has the drawback that it will agglutinate, sometimes immediately, in the blood, but this difficulty seems to be overcome by the use of graphite ink prepared according to the technique of Drinker and Churchill (1927).

The material for this is the preparation "Hydrocollag 300" to be obtained from E. de Haën, Seelze bei Hannover. The black syrup is mixed with twice its volume of water, made alkaline by the addition of sodium hydroxide (to  $p_H$  8.5) and the ammonia driven off by an air blast. To get rid of the aggregates which the preparation contains it is allowed to settle in a narrow cylinder for at least 24 hours in the ice chest. The up-

per portions are siphoned off and the few remaining oversize particles can now be removed by filtration. The alundum crucible R.A. 98. Norton Co., Worcester, Mass., U.S.A., has pores of just the right size. It can be cleaned by firing in the blast flame. Other filters or a centrifuge can no doubt be used and a greatly lengthened period of settling would bring about the same separation. This suspension can be mixed with salts, buffers, gum acacia, or serum without causing agglutination, but it will agglutinate slowly within the vessels and cause clotting of the blood.

Since the liver of a living animal will remove the particles at a rapid rate the animal should be suddenly killed as soon as the injected fluid has been completely mixed with the circulating blood. A few minutes will be necessary and sufficient for this. Care must be taken not to stimulate the capillary systems by the process of killing. For small animals I would recommend a blow on the head followed immediately by freezing. For many purposes the animal can be killed by a blow and the organs which it is desired to study immediately flooded with a fixing solution. The vitally injected preparations can be treated just like total injections, and studies in which stimulated and working organs are quantitatively compared with the same organs in a resting condition and with total injections are sure to give valuable information.

#### *The histology of the capillary wall.*

The endothelial cells and their border lines can be shown by silvering. The vessels are washed out with saline, and a dilute solution (0.2 per cent) of argentine is injected. After  $\frac{1}{4}$  to  $\frac{1}{2}$  hour the excised thin membranes are exposed on slides to the light until the black silver lines become distinct, or the silver is reduced by a very dilute solution of one of the photo-



graphic developers in which the preparation is placed until it begins to look brown. The epithelium is removed by means of a brush and the preparation is mounted in glycerine.

The smooth muscle cells can be observed in their natural living state on young newt larvae by the technique described (p. 369).

For supravital staining the fresh nictitating membrane of a frog is placed for a period of 6-12 hours in 0.1 per cent methylene blue dissolved in a 0.7 per cent saline solution. The preparation can be fixed in a saturated solution of ammonium picrate (6-12 hours), and, after removal of the epithelium from both surfaces, examined in a mixture of this fluid with an equal volume of glycerine, in which it can be permanently mounted.

To obtain an elective staining of myofibrils according to Bensley and Vimtrup (1928) the vessels of a frog are washed out with Ringer's solution and injected with janus green B (Meister, Lucius, and Brünning) 1-15,000 in Ringer. After 10-15 minutes a 5 per cent solution of ammonium tungstate is injected to fix the stain. It is safer, however, to place the nictitating membrane or the web under the microscope five minutes after the injection of the janus green and to observe the progressive staining and reduction of the dye. The endothelium is the first to show a blue stain deepened in the nuclei. Thereupon, the cell borders and the nerves become bluish. These elements lose the colors in a few minutes, and the muscles cells become stained with a blue-violet tint, changing gradually to purple and concentrating in the myofibrils. The change and gradual disappearance of the color are caused by a breakdown of the dye brought about by the living tissue in the absence of oxygen. The reaction is brought to a standstill and fixed at the suitable stage by am-

monium tungstate. The preparation remains in the fixing solution for 2-24 hours and is studied in a mixture of it with equal parts of glycerine. To make the preparation permanent it is dehydrated in ice-cold alcohol treated with ice-cold xylol and mounted in balsam.

The capillary nerves can be vitally or supravitaly stained according to Busch in tissues from cold- or warm-blooded animals by means of methylene blue (0.01 per cent solution in Ringer) or rongalit white (0.005 per cent) introduced by injection or infiltration. The membranes or small pieces of tissue excised for observation are placed in the same dye solution and saturated with oxygen. They should remain under 1 atm. oxygen pressure in an incubator at 35°-40° for 15-30 minutes according to the thickness of the piece. Thin membranes are studied within an hour, or the preparation is fixed by means of saturated ammonium picrate as above described. The fixed tissue can be cut into sections in a frozen condition and cleared and mounted in a mixture of equal parts of ammonium picrate and glycerine.

#### *Stimulation of capillaries.*

Stimulation of capillaries can be carried out in so many ways that a general description of methods cannot be given, though it may be useful to note a few points.

The local effect of gases on tissue membranes can be tested by means of the apparatus shown in Lecture VIII, p. 172. Soluble substances including gases can be added to the Ringer solution irrigating the tissue, but when gases are used it is necessary to irrigate under a cover glass to prevent gas exchange with the atmosphere.

When working without irrigation, one places reagents on the tissue under the microscope by means

of a glass rod ending in a knob, the size of which will determine approximately the size of drop delivered. Dilute solutions acting over a larger but well-defined area are placed in reaction basins, open rings of suitable dimensions (e.g., outside diameter 4 mm., inside diameter increasing from 2 to 3.5 mm., height 1 or 1.5 mm.) placed on the surface and filled with the solution in question. Almost any metal will have some action on the tissue and capillaries and we now make the reaction basins from ebonite.

To introduce substances into the human skin the most suitable method is generally to place a small drop on the surface and prick through it with a fine needle (Th. Lewis). Many substances can be conveniently introduced into a larger area by kataphoresis (Ebbecke, 1923).

#### *Perfusion methods.*

The study of capillary reactions by artificial perfusion has at present only a very limited field of usefulness, but there are cases where it must be resorted to. It is to be emphasized, because often forgotten, that the ordinary methods of recording the perfusion flow are useless for our purposes. They record the state of arterioles, but say nothing of the capillaries. Changes in volume of a perfused limb or organ may be due either to changes in the volume of the capillaries or to passage of fluid through their walls, and when the changes are small it may be difficult to distinguish in the experiment between these mechanisms. Perfusion fluids must possess the normal freezing point of plasma and be ionically balanced so as not to damage the tissues. They must contain a colloid substance which will maintain an effective osmotic pressure of suitable magnitude. Serum proteins or purified gum acacia are suitable for this purpose. They must be

able to carry the necessary oxygen to the tissues, and in experiments on warm-blooded animals the addition of red corpuscles will be necessary to insure this, while in cold-blooded forms the same end can be attained by saturation with oxygen. When changes in capillary diameter are the object of study the fluid must be suitably stained either with a colloid dye or with a suspension of very small particles which will leave no zone of clear plasma along the vessel walls.

Graphite suspensions as well as clear solutions can be perfused continuously, but when corpuscles are added rhythmic perfusion combined with an arrangement for stirring the fluid in the container becomes necessary, and we have got the impression that rhythmic perfusion is generally to be preferred. A diagram of my apparatus for rhythmic perfusion with fluids containing corpuscles is shown in Fig. 99. Air (or any other gas or gas mixture) is supplied along the tube (1) at a suitable pressure. The cylinder (2), in which the tube (3) can be raised and lowered, constitutes a pressure regulator, the surplus of air supplied being blown off through the mercury (or water). (4) is a metal tap turned at a regular rate, corresponding to the pulse rate, by a small motor. When the tap is in the position shown, the compressed air is admitted to a vessel (5) (in reality a series of vessels) and when the tap is turned through  $180^\circ$  the air is blown off through the tube (6), and the fluid resistance (7). We obtain at each cycle a definite systolic pressure, regulated by the resistance in (2), and a diastolic pressure, regulated by (7). The rubber stopper of each perfusion vessel is perforated by three glass tubes: (8) is a T tube admitting the compressed air and serving further for the introduction of perfusion fluid, (9) and (10) are screw clips. The tube (11) is provided with a glass bulb, closed at the top and containing air. At each pulse

the perfusion fluid is driven up into the tube (11) and back again, thereby keeping the perfusion fluid constantly and completely mixed. The narrow tube (12) can be connected through suitable rubber tubing with

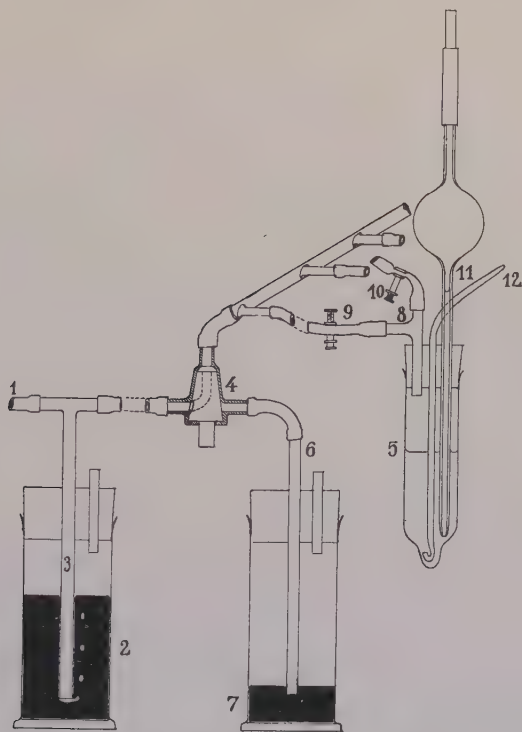


Fig. 99. Apparatus for rhythmical perfusion.

the perfusion cannula. When a T tube is inserted just in front of the cannula and connected with a manometer the effective perfusion pressure can be measured.

Drinker (1927) describes certain modifications which are useful for measuring the rate of perfusion and quickly changing from one fluid to another.



*The rate of diffusion through tissue membranes.*

The apparatus, Fig. 100 (Krogh, 1919), has been used to determine the diffusion of gases. It consists of two metal chambers, *A* and *B*, of 1.5 cc. and about 50 cc. capacity, respectively. A piece of suitable membranous tissue, as, for instance, a thin flat muscle, is arranged as a partition wall between the two chambers, which are filled with blood. The mixing screws, 7 and 8, are arranged to secure the completest possible renewal of

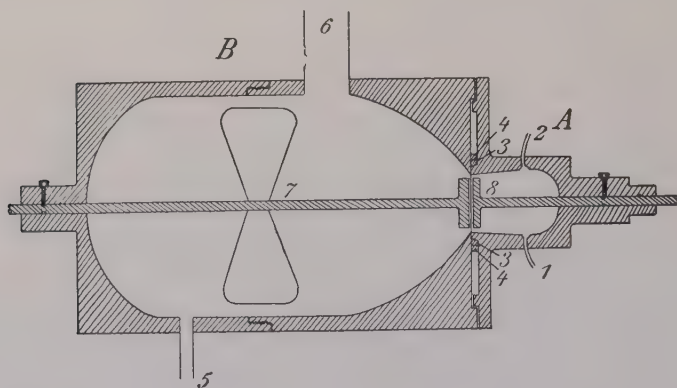


Fig. 100. Apparatus for measuring diffusion through tissue membranes.

the fluid along both surfaces of the membrane. The blood in *B* is saturated with oxygen at one atmosphere pressure, while that in *A* is oxygen-free. The oxygen passing through the membrane enters at once into combination with the hemoglobin, and its quantity can be determined after a suitable period of diffusion. When the area and thickness of membrane employed are also determined the diffusion constant can be calculated.

The important point in measuring the rate of diffusion of dissolved substances from one fluid to another through a membrane is to secure complete mixture of

the fluids right up to the surfaces of the membrane. If there is a resting layer of fluid the substance will have to diffuse through this also, and in case of a thin membrane and a slow diffusion through the fluid the rate measured may become a great deal too low.

*Determination of colloid osmotic pressure.*

The solution containing the colloid or colloids is enclosed in a vessel in which the pressure can be read off and very small changes in volume detected. Part of the wall of the vessel is made up of a membrane which is impermeable to the colloids in question but permeable to crystalloids. On the outside of the membrane a solution is placed containing the same crystalloids as the inner solution. When equilibrium has been attained the pressure of the inside fluid is regulated until the volume remains constant, that is, until the filtration of water through the membrane balances exactly the osmotic attraction of water, and this pressure is the osmotic pressure sought.

The important point is to attain equilibrium and to make sure that it has been attained. As it is usually impracticable to obtain an outside fluid which is in equilibrium beforehand a certain amount of diffusion must take place through the membrane during the experiment. To facilitate this the membrane must be as large as possible in proportion to the volume of inside fluid; it must be as permeable as possible, i.e., just impermeable to the colloids in question and, further, the volume of outside fluid must be as small as possible, which insures in addition that the unavoidable change in composition of the inside fluid is minimized.

The first and third of these conditions can be fulfilled by the construction of the osmometer, and generally it can be said that the smaller the volume the

larger in proportion will be the surface exposed through the membrane.

The membranes must be tested for protein impermeability either by a dialysis experiment of at least twenty-four hours duration with the smallest possible quantity of outside fluid or preferably by ultra-filtration of serum under pressure. The ultrafiltrate or outside fluid is tested qualitatively for protein. Heller's test is scarcely sensitive enough, but a corresponding test with Spiegler's solution (4 g. mercuric chloride, 2 g. tartaric acid, 10 g. glycerine in 100 cc. distilled water) is very satisfactory. The Spiegler test should be negative or indicate at most the faintest trace of protein.

Membranes which just pass the protein test may have very different permeabilities for water and crystalloids. The protein test shows, according to present conceptions, that there are no pores (or a negligible number) above a certain size. It is essential that there should be the largest possible number of smaller pores. This is tested by a determination of the rate of filtration of water through a definite area under a definite pressure. I have defined the filtering capacity as the number of cc. filtering per minute through 100 cm.<sup>2</sup> at 1 atm. pressure. For the Zsigmondy filters the "minute number" is given as the time required for the filtration of 100 cc. through 100 cm.<sup>2</sup> The minute number  $N$  is, therefore, inversely proportional to the filtration rate  $F$  and we have  $N = \frac{100}{F}$  or  $F = \frac{100}{N}$ .

The best membranes at present available are colloidion membranes prepared according to Walpole (1915, Krogh and Nakazawa, 1927), and especially the flat membranes prepared according to Zsigmondy and obtainable from Dr. Kratz, Göttingen. Filtering

capacities from 0.8 to 1.4 (minute numbers from 125 to 70) can be obtained with membranes impermeable to protein. Parchment membranes or collodion membranes prepared according to Brown (1915) show much lower filtering capacities.

Several types of osmometer are in use which fulfil the conditions given above, and I refer only to the form specially constructed for clinical use (Krogh and Nakazawa) and to be obtained with suitable mem-

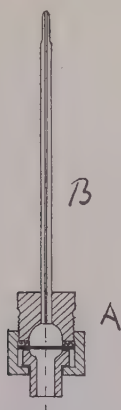


Fig. 101. Osmometer for clinical use.  
After Krogh and Nakazawa.

branes from Dr. Kratz; it is shown in Fig. 101. With regard to the technical details I refer to the published descriptions and would emphasize only the necessity of excluding bacterial growth which usually brings about a continuous decrease in the osmotic pressure observed.

#### *Determination of capillary and venous pressure.*

The standard method of determining the pressure of a fluid at a given point in a system of tubes or blood

vessels is to connect it at the desired point with a manometer, the simplest form of which is a vertical tube filled with the fluid of the system. This holds no less for the capillaries than for any other blood vessels and the indirect methods to be described can be considered as safe only in so far as they have been checked by comparison with the standard method under strictly comparable conditions.

In the direct method of determining the pressure in minute blood vessels as worked out by Landis (1926) the vessel and the surrounding tissue are pierced by a micro-pipette drawn from hard glass (Pyrex capillary tubing) into a short tip 4-8 micron in diameter. The pipette is mounted in a micro-manipulator and connected through an elastic spiral with a manometric system. The whole is filled with water, and devices are provided for changing the level of the manometer and for moving the fluid in the micro-pipette independently of the manometer. For pressure measurements a highly colored dye solution is taken into the pipette. To avoid clogging of the tip, solutions to be used for this purpose are sharply centrifuged in sealed tubes to be opened only just before the solution is to be sucked into the micro-pipette (Landis I, 1927). So far this method has been used only on the frog's mesentery, but it should be adaptable to many other tissues, including the skin of man.

The pressure, which it is normally desired to measure, is the side pressure with a perfectly undisturbed flow. Theoretically this can be done only when the tip of the pipette is at a right angle to the vessel and just piercing the wall. In practice this condition is difficult or impossible to fulfil and some obstruction is generally unavoidable. When the pipette is inserted into a vessel forming part of a network, so as to obstruct the flow completely the obstructed vascular branch



becomes part of the manometer and the pressure is measured in the vessel from which it branches. This measurement gives the true side pressure when the branching is at a right angle. With the slow flow in capillaries and venules the error introduced by angles between  $45^\circ$  and  $135^\circ$  will be negligible.

On account of the difficulties inherent in inserting manometric appliances in minute vessels a number of indirect methods for determining capillary pressure have been worked out. In some of these a small glass plate is pressed against the tissue (usually the human skin) and the force measured which is necessary to blanch the skin or compress the microscopically visible vessels. The force expressed in gm. per sq. cm. is supposed to give the pressure in the vessels compressed. There are very serious sources of error in this technique, one of which is that the area compressed is always larger to an indefinite extent than the plate employed. In a recent modification of this method Rajka and Wessely (1923) use a convex plate, mounted in the focus of the objective of a microscope which is in turn mounted on a balance to compress the vessels of the human skin. The pressure exerted is expressed in gm. but cannot be referred to any area, and the figures obtained bear, therefore, no relation whatever to the capillary pressure in the accepted sense of the term.

The error arising from the lack of definition of the area under pressure can be avoided by applying a pressure capsule—a metal ring closed on one side by a glass window for inspection and on the other by a transparent, soft, but unelastic, membrane. The capsule is connected with some arrangement for varying and measuring the air pressure within, and this is obviously the same as the maximum pressure which can be exerted by means of the convex surface of the membrane. When the membrane is applied to the skin

and the pressure in the capsule raised, until the skin under it blanches conspicuously, a measurement of capillary pressure is taken to be obtained (Kylin, 1920, 1921).

One objection to this technique is derived from the fact that the necessary deformation of the elastic skin and subcutaneous tissue takes up a certain, unknown fraction of the pressure which is not transmitted to the vessels at all. This error will make measurements over soft parts of the body illusory, but it can be overcome to a certain extent by selecting places where the skin is directly supported by bone (knuckles, head of ulna, kneecap).

Another and much more serious objection to all compression methods involving more than a single vessel is the lack of definition of the kind of vessel on which the determination is really made.

Supposing that we could compress by a capsule a single capillary loop, so as to stop the blood flow through it, the pressure required would obviously be that obtaining at the point of branching of that loop from its arteriole. When in actual practice all the capillaries over an area of several sq. mm. are compressed to obliteration nobody can tell the level on the arterial side represented by the measurement, but it certainly must be farther up than the terminal arterioles. Leonard Hill (1920, 1921), who has emphasized this difficulty, has attempted to get over it by taking as his index instead of the complete obliteration of the vessels under pressure the slightest diminution in diameter to be observed microscopically, and macroscopically the just visible blanching. In this he has been followed by Krogh and Rehberg (1927) who observed, however, a "distinct" blanching to make sure that the pressures recorded were if anything too high, but Klingmüller (1925) and Lewis (1927, 2) have

shown that capillary pressures measured in this way may be considerably lower than the pressures used in an armlet to obstruct the venous outflow. Since this is physically impossible it follows that the determinations are in error. Landis (1926) has shown further that the determinations of Leonard Hill on the frog's mesentery give too low values. The explanation of the discrepancy is to be found in the elastic properties of the vessel wall and, to a certain extent, of the tissues generally. When an elastic tube is expanded by an inside pressure until equilibrium is reached, any pressure exerted from outside will disturb that equilibrium and cause some contraction. It is obvious, therefore, that neither complete obliteration nor the just visible blanching can be used as an index in capillary pressure measurement by means of a capsule.

Lewis (1927, 2) has tested the capsule pressure measurements by throwing the same pressure on a capsule and on an armlet obstructing the venous outflow and observed that the degree of blanching as compared with the congested but uncompressed skin rises with increasing pressure while approximately the same endpoint, namely, the original uncongested color of the skin, is reached in each case. He maintains, therefore, that blanching to this endpoint will give a truer index than distinct blanching and one which cannot exceed the actual value. This is probably so when the blood flow remains constant or is reduced by venous obstruction, but, when the flow is greatly increased by arteriolar dilatation without any venous congestion and the venules dilated, blanching to this endpoint must give too high results, even disregarding the fact that the blood becomes arterialized and the true endpoint, therefore, must correspond to a skin color which is distinctly redder than the original, and disregarding further the difficulty of accurately judging the skin color through the capsule.

Rehberg and I have attempted to utilize capillary pulsation for pressure testing, but this can be done only in cases where this reaction can be made distinctly visible. Macroscopic "capillary" pulsation depends upon large variations in the diameter especially of the venules. Just as in arteries such variations attain their maximum when the outside pressure exceeds the minimum pressure during diastole, but falls short of the pressure during the passage of the systolic wave. In measurements of capillary pressure by means of this method the lowest pressure at which capillary pulsation can be definitely observed is to be taken as the mean venule pressure. At higher pressures the capillary loops and arterioles will be progressively compressed during diastole to be opened up with the venules during systole, and the "capillary pulsation" will become even more distinct, until a pressure is reached which diverts most of the blood through the arterial anastomoses out of the compressed area.

It might be argued that even in these determinations the capsule pressure may possibly be lower than the actual vascular pressure on account of the elasticity of the vessels. This seems unlikely, because the difference between systolic and diastolic pressure in the venules must be slight, and considerable variations in their diameter are consequently to be expected only when the outside pressure is very nearly the same as the internal. In any case the elasticity can only act so long as the vessels are passively dilated beyond their normal diameter, but the objection can only be definitely disposed of by comparative determinations. In such determinations of the pressure in reflex flares produced over the head of the ulna one person observed the field through the capsule and indicated the appearance and disappearance of capillary pulsation, while a second person regulated and noted the corre-

sponding capsule pressure which was finally compared with the armlet pressure, regulated by a third person and unknown to the other two. In one such series on the same histamine flare, pulsation was observed at capsule pressures of 39, 29, 20, 15, and 13 cm. water but disappeared at 10 cm. The pressure in the armlet was 0 and we take the result to indicate the venule pressure with an unobstructed flow. In the following series we found:

Pulsation at 30-29 cm.	No pulsation at 27 cm.	Armlet pressure 28.
Pulsation at 45, 42 cm.	No pulsation at 39 cm.	Armlet pressure 38.5
Pulsation at 25 cm.	No pulsation at 22 cm.	Armlet pressure 16.
Pulsation at 19, 18 cm.	No pulsation at 17 cm.	Armlet pressure 0.

These results agree with the expectation that at high armlet pressures, which obstruct the flow, the venule pressure should be practically the same as that of the larger veins, while at vein pressures near zero the rate of flow should correspond to a definite pressure head from the venules to the larger veins.



## BIBLIOGRAPHY

The lectures in which the papers quoted have been referred to are indicated within parentheses at the end of each entry.

- ABEL, J. J., and GEILING, E. M. K. (1924). Some hitherto undescribed properties of the constituents of Witte's peptone. *Journ. Pharm. and Exper. Therap.* **23**, 1-27. (9, 10.)
- ADAIR (1926). Application of Dalton's law of partial pressures. *Physiol. Congress Stockholm*. (12.)
- ALBERT, F. (1924). Contribution a l'etude des troubles vaso-moteurs "reflexes" d'origine traumatiques. Thèse de Liege. (7.)
- AREY and SIMONDS (1920). *Anat. Record*, **18**, 219. (11.)
- ASCHOFF (1924). *Lectures on Pathology*. New York. (4.)
- ASCHOFF (1924). Das reticulo-endotheliale System. *Ergebn. d. inn. Med.* (4.)
- ASHER UND SCHNEIDER (1926). Die Wirkung von Hormonen auf den Kapillarkreislauf unter möglichst physiolog. Bedingungen. *Biochem. Zeitsch.* **173**, 116. (9.)
- BAER, R. (1926). Ueber die Bedeutung d. quergestreiften Muskulatur für die Regelung des Wasserhaushaltes. *Arch. f. exp. Path. u. Pharm.* **119**, 102-118. (11.)
- BAER UND RÖSSLER (1926). Beitr. z. Pharmakol. d. Lebergefäße I. *Arch. f. exp. Path. u. Pharm.* **119**, 204-221. (11.)
- BARBOUR, H. (1921). The heat regulating mechanism of the body. *Physiol. Reviews*, **1**, 295. (7.)
- BARCROFT, J. (1908). Mechanism of vasodilatation in cat's submaxillary gland. *Proc. Physiol. Soc. Journ. Physiol.* **36**. (8.)
- BARCROFT, J. (1914). *The respiratory function of the blood*. Cambridge Univ. Press. (2.)
- BARCROFT, J. (1926). Die Stellung der Milz im Kreislaufsystem. *Ergebn. d. Physiol.* **25**, 818-861. (11.)
- BARCROFT, J., and KATO, T. (1915). Effects of functional activity in striated muscle and the submaxillary gland. *Phil. Trans. Roy. Soc. London. Ser. B.* **207**, 149. (8, 12, 13.)
- BARCROFT, J., and STEPHENS, J. C. (1927). Observations upon the size of the spleen. *Journ. Physiol.* **64**, 1. (11.)
- BARDY, H. (1918). Ueber Hemmung inflammatorischer Symptome. *Skand. Arch. Physiol.* **32**, 198. (6.)
- BARGER, S., and DALE, H. H. (1911).  $\beta$ -iminazolyethylamine, a depressor constituent of intestinal mucosa. *Journ. Physiol.* **41**, 499. (9.)
- BARSDALE, J. (1925). The effect of dimethyl-guanidinsulphate upon the capillaries. *Southern Med. Journ.* **18**, 707. (8.)

- BASLER, A. (1914). Untersuchungen über den Druck in den kleinsten Blutgefäßen der menschlichen Haut. *Pfl. Arch.* **157**, 345. (13.)
- BAYLISS, W. M. (1901). On the origin from the spinal cord of the vaso-dilator fibers of the hind limb and on the nature of these fibers. *Journ. Physiol.* **26**, 173. (5.)
- BAYLISS, W. M. (1902). Further researches on antidromic nerve impulses. *Journ. Physiol.* **28**, 276. (5.)
- BAYLISS, W. M. (1902). On the local reactions of the arterial wall to changes of internal pressure. *Journ. Physiol.* **28**, 220-231. (10.)
- BAYLISS, W. M. (1916). Viscosity and intravenous injection of saline solutions. *Journ. Physiol.* **50**, 23. (15.)
- BAYLISS, W. M. (1919). The action of gum acacia on the circulation. *Journ. Pharm. and Exper. Ther.* **15**, 29. (15.)
- BAYLISS, W. M., and STARLING, E. H. (1894). Observations on venous pressures and their relationship to capillary pressures. *Journ. Physiol.* **16**, 159. (13.)
- BEALE, L. P. (1860). On the distribution of nerves to the elementary fibers of striped muscle. *Phil. Trans. Roy. Soc. London*, **1860**, 611. (5.)
- BENNINGHOFF, A. (1926). Ueber die Formenreihe der glatten Muskulatur und die Bedeutung der Rouget'schen Zellen an den Kapillaren. *Zeitsch. f. Zellforsch. u. mikr. Anat.* **4**, 125-170. (4.)
- BENSLEY, R. R., and VIMTRUP, B. (1928). Undersøgelser over de Rougetske Cellers Funktion og Struktur. *Danske Videnskabernes Selskab. Biol. Medd.* **7**, 1-26.—On the nature of the Rouget Cells (to appear in the *Anatomical Record*, 1928). (4, 4.)
- BERNARD, CLAUDE (1852). Sur les effets de la section de la portion céphalique du grand sympathique. *C. R. Soc. Biol.* **4**, 168. (15.)
- BEST, C. H., DALE, H. H., DUDLEY, H. W., and THORPE, W. V. (1927). The nature of the vaso-dilator constituents of certain tissue extracts. *Journ. Physiol.* **62**, 397-417. (9.)
- BIEDL UND KRAUS (1909). Experimentelle Studien über Anaphylaxie. *Wien, klin. Woch.* **1909**, No. 11. (15.)
- BIER, A. (1897). Die Entstehung des Collateralkreislaufs, I. *Arch. f. pathol. Anat. und Physiol.* **147**, 256, 444. (10, 11.)
- BIER, A. (1898). Die Entstehung des Collateralkreislaufs, II. *Arch. f. pathol. Anat. und Physiol.* **153**, 306. (11.)
- BLOOM, W. (1922). Histamine as an inflammatory agent. *Johns Hopkins Hosp. Bull.* **33**, 185-188. (15.)
- BRASOL, L. von (1884). Wie entledigt sich das Blut von einem Ueber-schuss von Traubenzucker. *Arch. (Anat. u.) Physiol.* **1884**, 211. (13.)
- BRESLAUER, F. (1918). Die Pathogenese der trophischen Gewebsschäden nach der Nervenverletzung. *Berliner klin. Woch.* **1918**, 1073-1079. (5, 6, 8.)
- BRESLAUER, F. (1919). Die Pathogenese der trophischen Gewebsschäden nach der Nervenverletzung. *Deutsche Zeitschr. f. Chirurgie*, **150**, 50. (5, 6, 8.)
- BRICKER, SUPONITZKAJA UND TSCHARNI (1926). Zur Frage der Bluter-

- satzflüssigkeit bei schweren Blutverlusten. *Zeitsch. exp. Med.* **48**, 451-471. (15.)
- BROWICZ, T. (1899). Ernährungswege in der Leberzelle nebst einem Résumé über die Resultate der seit 1897 in den Publikationen der Akademie veröffentlichten Untersuchungen des Verfassers über die Leberzelle. *Anz. Akad. Wiss. Krakau*, **1899**, 359-365. (4.)
- BROWN, G. E., and GIFFIN, H. Z. (1926). Studies of the vascular changes in cases of polycythemia vera. *Amer. Journ. Med. Sciences*, **171**, 157. (11.)
- BROWN, G. E., and SHEARD, CH. (1926). Measurements on the skin capillaries in cases of polycythemia vera and the rôle of these capillaries in the production of the erythrosis. *Journ. Clin. Investig.* **2**, 423-434. (11.)
- BROWN, W. (1915). On the preparation of collodion membranes of differential permeability. *Biochem. Journ.* **9**, 591. (4.)
- BRUCE, A. N. (1910). Ueber die Beziehung der sensiblen Nervenendigungen zum Entzündungsvorgang. *Arch. f. exper. Path. u. Pharm.* **63**, 424. (6.)
- BRUCK, C. (1909). Experimentelle Beiträge zur Aetiologie und Pathogenese der Urticaria. *Arch. f. Dermat. u. Syphilis*, **96**, 241. (5, 14.)
- BRUNS UND KÖNIG (1920). Ueber die Strömung in den Blutkapillaren der menschlichen Haut bei kalten und warmen Bädern und über die Reaktion in und nach kühlen Wasser- und Kohlensäurebädern. *Zeitschr. f. physikal. und diätet. Therapie*, **24**, 1. (3.)
- BRÜCKE, E. TH. (1927). L. A. Orbelis Untersuchungen über die sympathische Innervation nicht vegetativer Organe. *Klin. Woch.* **1927**, No. 15. (7.)
- BURN, J. H. (1922). The relation of nerve supply and blood flow to sweating produced by pilocarpine. *Journ. Physiol.* **56**, 232. (9.)
- BURN, J. H., and DALE, H. H. (1926). The vaso-dilator action of histamine, and its physiological significance. *Journ. Physiol.* **61**, 185-214. (9.)
- CARRIER, E. B. (1922). The reaction of the human skin capillaries to drugs and other stimuli. *Amer. Journ. Physiol.* **61**, 528-547. (8, 9, 11.)
- CARRIER, E. B. (1926). Observation of living cells in the bat's wing. *physiol. Papers. Dedicated to Prof. A. Krogh, Levin & Munksgaard Copenhagen*, **1926**, 1-9. (4.)
- CARRIER, E. B., and REHBERG, P. B. (1923). Capillary and venous pressure in man. *Skand. Arch. Physiol.* **44**, 20-31. (13.)
- CEVARIO, L. (1921). Sulla patogenesi della morte per ustione. *Pathologica*, **13**. (15.)
- CHAUVEAU, A., ET KAUFMANN, M. (1887). Expériences pour la détermination du coefficient de l'activité nutritive et respiratoire des muscles en repos et en travail. *C. R.* **104**, 1126. (8.)
- CHIARI, R., UND JANUSCHKE (1910). Hemmung von Transsudat- und Exsudatbildung durch Kalziumsalsze. *Wien. klin. Woch.* **23**, No. 12. (14.)

- CHIARIELLO, A. G. (1925). Contributo alle studio della fine istologia dei capillari. *Ann. ital. di chir.* **4**, 888-899. (4.)
- CHURCHILL, NAKAZAWA, and DRINKER (1927). The circulation of body fluids in the frog. *Journ. Physiol.* **63**, 304-308. (12, 13.)
- CLARK, A. J. (1921). Absorption from the peritoneal cavity. *Journ. Pharm. and Exper. Therap.* **16**, 415. (12, 13.)
- CLARK, E. R., and CLARK, E. C. (1925). A. The development of adventitial (Rouget) cells on the blood capillaries of amphibian larvae. *Amer. Journ. Anat.* **35**, 239-264. (4.)
- CLARK, E. R., and CLARK, E. C. (1925). B. The relation of Rouget's cells to capillary contraction. *Amer. Journ. Anat.* **35**, 265-282. (4.)
- CLARK, E. R., and CLARK, E. C. (1926). The fate of extruded erythrocytes. *Amer. Journ. Anat.* **38**, 41. (1.)
- COHEN, APPLEBAUM, and HAINSWORTH (1926). Intracutaneous salt solution test. *Journ. Am. Med. Ass.* **86**, 1677. (14.)
- COHNHEIM, J. (1867). Ueber Entzündung und Eiterung. *Arch. f. path. Anat.* **40**, 1-80. (1, 3.)
- COHNHEIM, J. (1872). Untersuchungen über die embolische Prozesse. Berlin. (10.)
- COHNHEIM, J. (1877). Vorlesungen über allg. Pathologie I. (1.)
- COHNSTEIN UND ZUNTZ (1888). Untersuchungen über den Flüssigkeitsaustausch zwischen Blut und Geweben. *Pfl. Arch.* **42**, 303. (1.)
- CONNOLLY (1926). Vasodilatation in Fundulus due to color stimulation. *Biol. Bull. of the Marine. Biol. Lab.* **50**, 207. (11.)
- COTTON, T. F., SLADE, J. S., and LEWIS, T. (1917). Observations upon dermatographism with special reference to the contractile power of the capillaries. *Heart*, **6**, 227. (3, 8.)
- CRAIGIE, E. H. (1921). The vascularity of the cerebral cortex of the albino rat. *Journ. Comp. Neurol.* **33**, 193-212. (2, 4.)
- CRAIGIE, E. H. (1924). Changes in vascularity in the brain stem and cerebellum of the albino rat between birth and maturity. *Journ. Comp. Neur.* **38**, 27-48. (2.)
- CRAIGIE, E. H. (1925). Postnatal changes in vascularity in the cerebral cortex of the male albino rat. *Journ. Comp. Neur.* **39**, 301-324. (2.)
- CRAWFORD, I. H. (1926). Observations on the capillary circulation in normal subjects. *Journ. Clin. Invest.* **2**, 351. *Rockefeller Studies*, **58**, 457. (1.)
- CRAWFORD, I. H., and ROSENBERGER, H. (1926). An apparatus for cinematographic observation of human skin capillaries. *Journ. Clin. Invest.* **2**, 343. *Rockef. Stud.* **58**, 449. (4.)
- CUSHNY, A. R. (1917, second ed. 1926). The secretion of the urine. (4.)
- DALE, H. H., and LAIDLAW, P. P. (1919). Histamine shock. *Journ. Physiol.* **52**, 355. (9.)
- DALE, H. H., and RICHARDS, A. N. (1918). The vasodilator action of histamine and of some other substances. *Journ. Physiol.* **52**, 110. (3, 9.)
- DENNIG, H. (1924). Zur Physiologie der periarteriellen Nerven. *Klin. Woch.* **3**. (5.)

- DESCAMPS, A. (1925). Le calcium imperméabilise-t-il les parois vasculaires? *Arch. intern. Physiol.* **25**, 64-73. (14.)
- DIETER, W. (1925). Ueber den Zusammenhang zwischen osmotischem Druck, Blutdruck insbesondere Kapillardruck und Augendruck nach neuen experimentellen und klinischen Untersuchungen. *Arch. f. Augenheilk.* **96**, 180-264. (12.)
- DOAN, CH. A. (1922). The capillaries of the bone marrow of the adult pigeon. *Johns Hopkins Hosp. Bull.* **33**, 222-226. (11.)
- DOAN, CH. A. (1922). The circulation of the bone marrow. *Contrib. to Embryology*, **14**, 27-45. (11.)
- DOI, Y. (1920). On the existence of antidromic fibers in the frog and their influence on the capillaries. *Journ. Physiol.* **54**, 227. (5, 9.)
- DONNAN (1924). Theory of membrane equilibria. *Chem. Rev.* **1**, 73. (12.)
- DREYER, G., und JANSEN, H. (1905). Ueber den Einfluss des Lichtes auf tierischen Geweben. *Mitteilungen aus Finsens Lichtinstitut*, **9**. (10, 15.)
- DRINKER, C. K. (1927). The permeability and diameter of the capillaries in the web of the brown frog (*R. temporaria*) when perfused with solutions containing pituitary extract and horse serum. *Journ. Physiol.* **63**, 249-269. (5, 9, 14, A.)
- DRINKER, C. K., and CHURCHILL, E. D. (1927). A graphite suspension for intravital injection of capillaries. *Proc. Roy. Soc.* **101**, 462-467. (A.)
- DRINKER, C. K., DRINKER, K. R., and LUND, CH. C. (1922). The circulation in the mammalian bone-marrow. *Amer. Journ. Physiol.* **62**, 1-92. (1, 11.)
- DRURY and JONES (1927). Observations upon the rate at which edema forms when the veins of the human limb are congested. *Heart*, **14**, 55-70. (15.)
- DUKE, W. W. (1924). Urticaria caused specifically by the action of physical agents. *Journ. Amer. Med. Ass.* **83**, 3-9. (10.)
- DUKE, W. W., and STOFER, D. D. (1922). A comparison of capillary and venous blood in pernicious anemia. *Arch. Intern. Med.* **30**, 94-98. (1, 2.)
- DUYFF und BOUMAN (1927). Ueber die Capillarisation einiger Kaninchenmuskeln. *Zeitsch. f. Zellforsch. u. mikr. Anat.* **5**, 596-614. (2.)
- DÖLLINGER (1821). *Denkschr. d. K. Akad. d. Wiss. München*, 1821. (Quoted from Stegemann.) (3.)
- EBBECKE, U. (1917). Die locale vasomotorische Reaktion (L. V. R.) der Haut und der inneren Organe. *Pfl. Arch.* **169**, 1-81. (3, 6, 8, 10, 11, 14.)
- EBBECKE, U. (1923). Ueber Gewebsreizung und Gefässreaktion. *Pfl. Arch.* **199**, 197-216. (7, 10, A.)
- EBBECKE (1923). Gefässreaktionen. *Ergebn. d. Physiol.* **22**, 401-494. (4, 9, 10.)
- EDMUNDS and JOHNSTON (1928). The circulatory collapse in diphtheria. *Journ. Amer. Med. Ass.* **90**, 441. (15.)
- ELLERMANN, V. (1913). Ueber Anwendung getrennter Pipetten und



- Mischgefäße bei der klinischen Blutzählung. Deutsch. Arch. f. klin. Med. **109**, 378-382. (1.)
- ELLERMANN, V., und ERLANDSEN, A. (1910). Eine neue Technik der Lencocytenzählung. D. Arch. f. klin. Med. **98**, 245-257. (1.)
- ELLINGER, A., und HEYMANN, P. (1921). Die treibenden Kräfte für den Flüssigkeitsstrom im Organismus. Arch. f. exper. Path. u. Pharm. **90**, 336. (12, 14.)
- ERBEN (1918). Ueber vasomotorische Störungen. Wien. klin. Woch. **1918**, No. 2. (11.)
- EUGLING, M. (1908). Untersuchungen über den peripheren Tonus der Blutgefäße. Pfl. Arch. **121**, 275. (5.)
- EYSTER (1926). Venous pressure and its clinical applications. Physiol. Rev. **6**, 281. (15.)
- FAHR and SWANSON (1926). "Effective" osmotic pressure of plasma proteins. Amer. Journ. Physiol. **76**, 201. (12.)
- FÄHRAEUS, R. (1921). The suspension stability of the blood. Acta. med. scand. **55**, 1. (1.)
- FÄHRAEUS, R. (1928). Die Strömungsverhältnisse und die Verteilung der Blutzellen im Gefäßsystem. Klin. Woch. **7**, 100. (1.)
- FARKAS (1927). Studien über koll. osm. Druck des Serums. Zeitsch. ges. exp. Med. **53**, 666-676. (12.)
- FAUST (1904). Ueber das Fäulnisgift Sepsin. Arch. f. exper. Path. u. Pharm. **51**, 248. (9.)
- FEDERIGHI (1927). The blood vessels of annelids. Proc. Nat. Acad. Sc. **13**, 639-642. (4.)
- FELDBERG, W. (1927). The action of histamine on the blood vessels of the rabbit. Journ. Physiol. **63**, 211-216. (9.)
- FERRATA (1925). Haematologica, **2**, 242. (4.)
- FERRIO, C. (1926). Il condotto tessuto reticolare, considerazione critiche ed osservazione. Monitore zool. italiano. **37**. (4.)
- FINSSEN, N. R. (1900). Neue Untersuchungen über die Einwirkung des Lichtes auf die Haut. Mitteilungen aus Finsens Lichtinstitut, **1**. (10.)
- FISCHER, L. (1927). Die Einwirkung des Adrenalins auf die Kapillaren der menschlichen Körperoberfläche. Zeitsch. f. Biol. **86**, 351-366. (11.)
- FLEISCH, A. (1921). Die Wasserstoffionenkonzentration als peripher regulatorisches Agens der Blutversorgung. Zeitsch. f. allg. Physiol. **19**, 269. (8.)
- FLETCHER, W. (1898). The vaso-constrictor fibers of the great auricular in the rabbit. Journ. Physiol. **22**, 259. (10. 11.)
- FLOREY, H. (1925). Capillary permeability. Proc. Journ. Physiol. **61**, 1. (14.)
- FLOREY, H. (1925). Microscopical observations on the circulation of the blood in the cerebral cortex. Brain. **48**, 43-64. (6, 8.)
- FLOREY, H. (1926). Observations on the resolution of stasis in the finer blood vessels. Proc. Roy. Soc. B. **100**, 269-273. (1, 15.)
- FLOREY, H. (1927). Reactions of, and absorption by lymphatics, with special reference to those of the diaphragm. (4, 15.)

- FLOREY, H. W., and CARLETON, H. M. (1926). Rouget cells and their function. *Proc. Roy. Soc. B*, **100**, 23-31. (4, 9.)
- FREY, E. (1919). Das Gesetz der Abwanderung intravenös injizierten Stoffes aus dem Blute und seine Verteilung auf Blut und Gewebe. *Pfl. Arch.* **177**, 110-156. (12.)
- FRÖHLICH und ZAK (1924). Mikr. Studien am peripheren Kreislauf v. Kalt- und Warmblütern. *Zeitsch. ges. exp. Med.* **42**, 41. (4.)
- FÜLLEBORN, F. (1925). Ueber die Durchlässigkeit der Blutkapillaren für Nematodenlarven. *Arch. f. Schiffs- u. Tropenhygiene*, **29**, Beiheft 3. (1.)
- GAARDER, T. (1918). Ueber den Einfluss des Sauerstoffdruckes auf den Stoffwechsel. *Biochem. Zeitsch.* **89**, 94. (12.)
- GABBE, E. (1926). Über die Wirkung der sympathischen Innervation auf die Zirkulation und den Stoffaustausch in den Muskeln. *Zeitsch. ges. exp. Med.* **51**, 728. (5.)
- GAD-ANDRESEN, K. S. (1921). Die Verteilung des Harnstoffes im Organismus. *Biochem. Zeitsch.* **116**, 266. (12.)
- GÄNSSLEN (1927). Einfluss veränderter Nahrung auf den peripherischen Gefäßabschnitt. *Klin. Woch.* **6**, 786-791. (11.)
- GASSER, H. P., ERLANGER and MEEK (1919). Studies in secondary traumatic shock. IV. The blood volume changes and the effect of gum acacia on their development. *Amer. Journ. Physiol.* **50**, 31-52. (15.)
- GEILING, E. M. K., and KOLLS, A. C. (1924). Pharmacological action of primary albumoses in unanesthetized dogs. *Journ. Pharm. and exper. Therap.* **23**, 29-43. (10.)
- GESSELER, H. (1921). Ueber die Gewebsatmung bei der Entzündung. *Arch. f. exper. Path. u. Pharm.* **91**, 366. (12.)
- GESSELER, H. (1922). Ueber die Gewebsatmung bei der vasomotorischen Reaktion. *Arch. f. exper. Path. u. Pharm.* **92**, 273. (12.)
- GLASER, W. (1920). Innervation der Blutgefäße in L. R. Müller. *Das vegetative Nervensystem*. Berlin, Springer. 1920. (5.)
- GOLDBLATT, H. (1926). Observations upon reactive hyperemia. *Heart*, **12**, 281-294. (10.)
- GOLDSCHIEDER und HAHN (1925). Ueber Dermographie *Deutsche Med. Woch.* **51**, No. 11-13. (14.)
- GOVAERTS, M. P. (1924). Recherches cliniques sur le rôle de la pression osmotique des protéins du sang, dans la pathogenie des œdèmes et de l'hypertension artérielle. *Bull. acad. roy. de med. de Belgique*, **1924**, 1-54. (12.)
- GOVAERTS, M. P. (1927). Influence de la teneur du serum en albumines et en globulines sur la pression osmotique des protéins et sur la formation des œdèmes. *Bull. de L'acad. Roy. de Med. de Belgique*, **13**, 356-374. (12.)
- GROSSER, O. (1902). Ueber arterio-venöse Anastomosen an den Extremitätenenden beim Menschen und den krallenträgenden Säugetieren. *Arch. f. mikr. Anat. u. Entw.* **60**, 191. (4.)
- GUGGENHEIM und HIRSCH (1926). Ueber den Nachweis latenter Edeme

- aus dem Verhalten intracutaner Quaddeln einer Normosallösung. *Klin. W.* **5**, 704. (14.)
- HÄGEN, H. (1927). Untersuchungen über den intracutanen Gewebedruck. *Zeitsch. f. d. ges. exper. Med.* **57**, 203. (13.)
- HÄGEN, W. (1921). Die Schwankungen im Capillarkreislauf. *Zeitsch. f. d. ges. exper. Med.* **14**, 364-405. (11.)
- HÄGEN W. (1922). Periodische, konstitutionelle und pathologische Schwankungen im Verhalten der Blutcapillaren. *Virchow's Arch.* **239**, 504-556. (11.)
- HALDANE, J. S. (1917). *Organism and environment as illustrated by the physiology of breathing.* Yale University Press. (12.)
- HALL, H. (1925). A study of the pulmonary circulation by the transillumination method. *Amer. Journ. Physiol.* **72**, 446. (11.)
- HAMBURGER, R. (1922). Ueber die Bedeutung der Kalium- und Calciumionen für das künstliche Oedem und für die Gefäßweite. *Biochem. Zeitschr.* **129**, 153. (14.)
- HARRIS, E., and MARVIN (1927). The innervation of mammalian capillaries by vasoconstrictor sympathetic nerves. *Heart*, **14**, 135-138. (5.)
- HARTMAN, EVANS, and WALKER (1928). The action of epinephrin upon the capillaries and fibers of skeletal muscle. *Amer. Journ. of Physiol.* **85**, 91-98. (8.)
- HARTMAN, EVANS, MALACHOWSKI, and MICHALEK (1928). Effect of sympathetic nerve stimulation upon the capillaries and fibers of skeletal muscles. *Amer. Journ. of Physiol.* **85**, 99-102. (5.)
- HASTINGS, C. (1820). *Treatise on inflammation of lungs with exp. inquiry on contractile power of blood vessels.* London, 1820. (3.)
- HAYMAN, J. M., JR. (1927). Estimations of afferent arteriolar and glomerular capillary pressure in the frog kidney. *Amer. Journ.* **79**, 389-409. (13.)
- HAYMAN, J. M., and STARR, I. (1925). Experiments on the glomerular distribution of blood in the mammalian kidney. *Journ. exp. Med.* **42**, 641-659. (11.)
- HEIDENHAIN, R. (1888). Beiträge zur Histologie und Physiologie der Dünndarmschleimhaut. *Pfl. Arch.* **43**, Supplementheft. (15.)
- HEIDENHAIN, R. (1891). Versuche und Fragen zur Lymphbildung. *Pfl. Arch.* **49**, 209. (12.)
- HEIMBERGER, H. (1925). Beiträge z. Physiologie d. menschl. Capillaren. *Zeitsch. f. d. ges. exp. Med.* **46**, 519-557. (1, 4, 7.)
- HEIMBERGER, H. (1925). Experimentelle Untersuchungen über den Mikrocapillarpuls beim Normalen. *Klin. Woch.* **4**, No. 47. (4.)
- HEIMBERGER, H. (1925). Ueber die Kontraktilität der kleinsten Venen. *Zeitsch. f. d. ges. exp. Med.* **48**, 179-184. (4, 8.)
- HEIMBERGER, H. (1926). Beiträge zur Physiologie der menschlichen Capillaren, II. Verhalten auf stumpfen mechanischen Reiz. *Zeitsch. f. d. ges. exp. Med.* **49**, 411-426. (8.)
- HEIMBERGER (1926). Beiträge z. Physiol. d. menschl. Capil. III. *Zeitsch. exp. Med.* **51**, 112-123. (4, 8.)

- HEIMBERGER (1926). Beiträge z. Physiol. d. menschl. Kapill. IV. Zeitsch. ges. exp. Med. **53**, 107-120.
- HEIMBERGER (1927). Contractile Funktion u. anat. Bau der menschl. Capillaren. Zeitsch. f. Zellforsch. u. mikr. Anat. **4**, 713. (4, 8.)
- HEIMBERGER, H. (1927). Beiträge z. Physiol. d. menschl. Kapillaren V. Färbeversuche am Kapillarendothel und die Lymphräume des Papillarkörpergewebes. Zeitsch. f. d. ges. exp. Med. **55**, 17-24. (4.)
- HELSTED, A. (1905). Bidrag til Læren om Dødsårsagerne ved Forbrænding. Danish Dissertation. (15.)
- HEMINGWAY and McDOWALL (1926). The chemical regulation of capillary tone. Journ. Physiol. **62**, 166-173. (8.)
- HENDERSON, L. J., BOCK, A. V., FIELD, H., and STODDARD, J. L. (1924). Blood as a physicochemical system. II. Journ. Biol. Chem. **59**, 379-431. (12.)
- HENDERSON, Y. (1908). Acapnia and shock. I. Carbon dioxide as a factor in the regulation of the heart rate. (With the collaboration of M. M. Scarborough, F. P. Chillingworth, and J. R. Coffey.) Amer. Journ. Physiol. **21**, 126. (9.)
- HENDERSON, Y. (1909). Acapnia and shock. II. A principle underlying the normal variations in the volume of the blood stream and the deviation from this principle in shock. Amer. Journ. Physiol. **23**, 345-374. (9.)
- HENDERSON, Y. (1910). Acapnia and shock. VII. Failure of the circulation. Amer. Journ. Physiol. **27**, 152-176. (9.)
- HENDERSON, Y., and HARVEY, S. C. (1918). Acapnia and shock. VIII. The veno-pressor mechanism. Amer. Journ. Physiol. **46**, 33-53. (9.)
- HENDRIX, B. M., and SWEET (1917). Amino-nitrogen and glucose in lymph and blood before and after the injection of nutrient solutions in the intestine. Journ. Biol. Chem. **32**, 299. (15.)
- HERRING, P. F., and SIMPSON, S. (1906). On the relation of the liver cells to the blood vessels and lymphatics. Proc. Roy. Soc. London, **78**, 455. (4.)
- HERZOG, F. (1925). Die Rolle d. Kapillaren bei der Blutstillung. Pfl. Arch. **207**, 476-487. (1, 11.)
- HERZOG, F. (1925). Beziehungen z. Dilatation, Durchlässigkeit und Phagocytose an den Kapillaren d. Froschzunge. Virch. Arch. **256**, 1-8. (4, 14.)
- HERZOG (1925). Endothelium der Froschzunge als Phagocyten und Wanderzellen. Zeitsch. ges. exp. Med. **43**, 79-94. (4.)
- HEUBNER, W. (1907). Ueber Vergiftung der Blutkapillaren. Arch. f. exper. Path. u. Pharm. **56**, 370. (3, 9.)
- HEUBNER, W. (1922). Physiologie u. Pharmakologie d. Blutkapillaren. Klin. Woch. **2**, No. 43, 44. (8.)
- HEUBNER, W. (1925). Zur Pharmakologie der Reizstoffe. Arch. f. exp. Path. u. Pharm. **107**, 129-154. (9.)
- HILL, LEONARD (1920). Capillary pressure (I, II). Proc. Physiol. Soc. Journ. Physiol. **54**. (4.)
- HILL, LEONARD (1921). The pressure in the small arteries, veins and

- capillaries of the bat's wing. *Proc. Physiol. Soc. Journ. Physiol.* **54**, (4.)
- HINZELMANN (1924). Die Eklampsie, p. 393. (1.)
- HIRSCHFELDER (1924). Vascular and capillary phenomena and supposed axon reflexes in development of edema in mustard oil conjunctivitis. *Amer. Journ. Physiol.* **70**, 507. (6.)
- HOFF, F. (1927). Ueber Dermographia elevata. *Zeitsch. f. d. ges. exp. Med.* **57**, 253-293. (14.)
- HOFF und LEUWER (1926). Exp. Untersuchungen über die Permeabilität der Kapillaren b. Menschen. *Zeitsch. f. exp. Med.* **51**, 1-15. (14.)
- HOGBEN, L. T., and WINTON, F. R. (1922). Studies on the pituitary. I. The melanophore stimulant in posterior lobe extracts. *Bioch. Journ.* **14**, 619-630. (9.)
- HOOKE, D. R. (1911). The effect of exercise upon the venous blood pressure. *Amer. Journ. Physiol.* **28**, 235. (13.)
- HOOKE, D. R. (1920). The functional activity of the capillaries and venules. *Amer. Journ. Physiol.* **54**, 30. (4, 5, 8, 9, 11.)
- HORIUCHI, K. (1924). Beiträge z. Frage der Venodilatatoren. *Pfl. Arch.* **206**, 473-480. (5.)
- HOUSSAY, B. A. (1918). La accion fisiologica de los extractos hipofisarios. Buenos Aires. (9.)
- HOYER, H. (1877). Ueber unmittelbare Einmündung kleinster Arterien in Gefäßäste venösen Charakters. *Arch. f. mikr. Anat.* **13**, 603. Tafel, 38-39. (4.)
- HUZELLA, TH. (1925). Der Mechanismus des Kapillarkreislaufs *Zeitsch. f. wiss. Biol.* **2**, 558-583. (3.)
- HÜFNER, G. (1897). Ueber die Bestimmung der Diffusionscoefficienten einiger Gase für Wasser. *Wiedemann's Annalen.* N. F. **60**, 134-168. (12.)
- IPSEN, J. (1927). Om Sympathieuskirurgi særlig den periarterielle Sympathectomi. *Bibl. f. Læger.* Juni 1927. (7.)
- ISAYAMA, S. (1924). Ueber die Strömung der Lymphe bei den Amphibien. *Zeitsch. f. Biol.* **82**, 91-99. (13.)
- ISAYAMA, S. (1924). Ueber die Geschwindigkeit des Flüssigkeitsaustausches zwischen Blut und Gewebe. *Zeitsch. f. Biol.* **82**, 101. (13.)
- ITO, T. (1926). Ueber den Flüssigkeitsaustausch zwischen Lymphe und Blut b. Frosche. *Pfl. Arch.* **213**, 748. (13, 14.)
- IVERSEN, P. (1928). Undersøgelser over Ascitespatogenese. *Ugeskrift for Læger*, **1928**, 575-579. (13, 15.)
- IVERSEN, P., and NAKAZAWA, F. (1927). Ueber die Biochemie des Filtrationsödems. *Biochem. Zeitsch.* **191**, 307-319. (12, 15.)
- JACOB, W. (1920). Beobachtungen am peripheren Gefäßapparat unter lokaler Beeinflussung desselben durch pharmakologische Agentien. *Arch. f. exper. Path. u. Pharm.* **86**, 49. (8.)
- JACOB, W. (1921). Pharmakologische Wirkungen am peripheren Gefäßapparat und ihre Beeinflussung auf Grund einer spezifischen Veränderung der Permeabilität der Zellmembranen durch Hydroxylionen. *Arch. f. exper. Path. u. Pharm.* **88**, 333. (8.)



- JANSEN, H. (1906). Experimentelle Studier over Finsen-Behandlingsens Virkemaade. Danish Dissertation. (10.)
- JARISCH und LUDWIG (1927). Ueber das Pfortadergebiet als Blutreservoir. Arch. f. exp. Path. u. Pharm. **124**, 102-117. (11.)
- JORES, A. (1927). Der Einfluss der Muskulatur auf den Füllungszustand der Kapillaren. Zeitsch. f. exp. Med. **59**, 172-181. (A.)
- KENDREW (1926). Graphic registration of venous pressure in man illustrated by some observations on reactive hyperemia. Heart, **13**, 101. (13.)
- KILLIAN, H. (1925). Untersuchungen über die Wirkung von Adrenalin, Hypophysen-extrakt und Histamin auf den Blutstrom in den kleinsten Gefässen der Froschzunge. Arch. f. exp. Path. u. Pharm. **108**, 255. (8, 9.)
- KLINGMÜLLER, M. (1925). Ueber Capillardruck. Capillarstudien. I. **46**. Zeitsch. ges. exp. Med. **47**, 244. (A.)
- KOHLER und WETH (1924). Die Wirkung der cervicalen Sympathektomie auf die Angina pectoris und die Ausfallerscheinungen nach diesen operativen Eingriff. Zeitsch. f. klin. Med. **99**, 205-231. (6.)
- KOLLS, A. Ö., and GEILING, E. M. K. (1924). Contributions to the pharmacology of extracts of the posterior lobe of the pituitary gland. Journ. Pharm. a. exp. Therap. **24**, 67-81. (9.)
- KREYBERG, L. (1927). Die Rolle der Blutgefässe in der Genese der Teertumoren. Zeitsch. f. Krebsforsch. **26**, 191-193. (11.)
- KREYBERG, L. (1927). On local alterations of the blood-vessels of tar-painted white mice. Brit. Journ. Exp. Path. **8**, 465-470. (11.)
- KRIMKE, A. (1884). Die Nerven der Kapillaren. Dissertation. München. (5.)
- KROGH, A. (1904). On the cutaneous and pulmonary respiration of the frog. Skand. Arch. Physiol. **15**, 328-419. (8.)
- KROGH, A. (1912). The regulation of the supply of blood to the right heart. Skand. Arch. Physiol. **27**, 227. (11.)
- KROGH, A. (1914). Ethyl urethane as a narcotic for aquatic animals. Internat. Revue f. Hydrobiol. **6**, 42-47. (9.)
- KROGH, A. (1918). Vævenes Forsyning med Ilt og Kapillærkredsløbets Regulering. Danske Vid. Selsk. Biol. Medd. **1**, No. 6. (3.)
- KROGH, A. (1919). The rate of diffusion of gases through animal tissues, with some remarks on the coefficient of invasion. Journ. Physiol. **52**, 391. (12, A.)
- KROGH, A. (1919). The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. Journ. Physiol. **52**, 405. (2, 3, 12.)
- KROGH, A. (1919). The supply of oxygen to the tissues and the regulation of the capillary circulation. Journ. Physiol. **52**, 457. (1, 3, 12.)
- KROGH, A. (1920). Studies on the physiology of capillaries. I. The reaction to stimuli and the innervation of the blood vessels in the tongue of the frog. Journ. Physiol. **53**, 399. (3, 5, 6, 8, 9.)
- KROGH, A. (1921). Fortsatte Studier over Kapillærernes Fysiologi (Danish). Danske Vid. Selsk. Biol. Medd. **3**, No. 3. (1, 6, 8, 9.)

- KROGH, A. (1921). Studies on the physiology of capillaries. II. The reactions to local stimuli of the blood vessels in the skin and web of the frog. *Journ. Physiol.* **55**, 412. (1, 6, 8, 9.)
- KROGH, A. (1922). *The Anatomy and Physiology of Capillaries*. Yale Press. (9.)
- KROGH, A. (1924). *Anatomie und Physiologie der Capillaren*, Springer. (9.)
- KROGH, A. (1926). The pituitary (posterior lobe) principle in circulating blood. *Journ. Pharm. and exp. Therap.* **29**, 177-189. (9.)
- KROGH, A., and HARROP, G. A. (1921). On the substance responsible for capillary tonus. *Proc. Physiol. Soc. Journ. Physiol.* **54**. (9.)
- KROGH, A., and HARROP, G. A. (1921). Some observations on stasis and edema. *Proc. Physiol. Soc. Journ. Physiol.* **54**. (1, 14.)
- KROGH, A., HARROP, G. A., and REHBERG, P. B. (1922). Studies on the physiology of capillaries. III. The innervation of the blood vessels in the hind legs of the frog. *Journ. Physiol.* **56**, 179. (5, 6, 9.)
- KROGH et REHBERG (1922). Sur l'influence de l'hypophyse sur la tonicité des capillaires. *C. R. Soc. Biol.* **87**, 461. (9.)
- KROGH, A., and REHBERG, P. B. (1924). Kinematographic methods in the study of capillary circulation. *Amer. Journ. Physiol.* **68**, 153-160. (9, A.)
- KROGH, A., and REHBERG, P. B. (1927). The active relaxation of capillaries and venules in "reflex flare." *Proc. Journ. Physiol.* **64**. (A.)
- KROGH, A., und NAKAZAWA, F. (1927). Beiträge zur Messung des kolloid-osmotischen Druckes in biologischen Flüssigkeiten. *Biochem. Zeitsch.* **188**, 241-258. (12, A.)
- KROGH, M. (1915). The diffusion of gases through the lungs of man. *Journ. Physiol.* **49**, 271. (4.)
- KUPFFER, C. v. (1876). Ueber Sternzellen in der Leber. *Arch. f. mikr. Anat.* **12**, 352-358. (4.)
- KUPFFER, C. v. (1899). Ueber die sogenannten Sternzellen der Säugetierleber. *Arch. f. mikr. Anat.* **54**, 254-288. (4.)
- KYLIN, E. (1920) (1921). Eine Modifikation meines Kapillardruckmessers sowie Referat der Secher'schen Nachuntersuchungen mit diesem Messer. *Zentralbl. f. inn. Med.* **41**, No. 29, **42**, No. 40. (A.)
- KYLIN (1926). *Die Hypertoniekrankheiten*. Springer, 1926. (13.)
- LANDIS (1926). Capillary pressure in frog mesentery as determined by micro-injection methods. *Amer. Journ.* **75**, 548-571. (13, A.)
- LANDIS, E. M. (1927). Micro-injection studies of capillary permeability. I. Factors in the production of capillary stasis. II. The relation between capillary pressure and the rate at which fluid passes through the walls of single capillaries. *Amer. Journ. Physiol.* **81**, 124-142, **82**, 217-238. (14, A., 14.)
- LANDIS, E. M. (1928). Micro-injection studies of capillary permeability. III. The effect of lack of oxygen on the permeability of the capillary wall to fluid and to the plasma proteins. *Amer. Jour. Physiol.* **83**, 528-542. (14.)

- LANDSBERG, M. (1921). Studien über den Chemismus der Resorption d. pleuritischen Exsudate Wien. Arch. f. inn. Med. **3**, 467. (15.)
- LANGLEY (1900). On axon reflexes in the preganglionic fibers of the sympathetic nervous system. Journ. Physiol. **25**, 364-398. (6.)
- LANGLEY, J. N. (1921). The autonomic nervous system. Part I. Heffer and Sons, Cambridge. (6.)
- LAQUEUR, E. (1919). Ueber künstlich erzeugtes (osmotisches) Lungenödem und über Resorption in der Lunge. München med. Woch. **1919**, 1721. (13.)
- LAQUEUR, E., und MAGNUS, R. (1921). Ueber Kampfgasvergiftungen. V. Experimentelle und theoretische Grundlagen zur Therapie der Phosgenerkrankung. Zeitsch. f. d. ges. exper. Med. **13**, 200. (14.)
- LAZAREW und LAZAREWA (1926). Ueber die funktionelle Veränderungen der Blutstrom nach Röntgenbestrahlung. Strahlentherapie, **23**, 45-78. (10.)
- LEATHES, J. B. (1895). Some experiments on the exchange of fluid between the blood and the tissues. Journ. Physiol. **19**, 1. (13.)
- LEBER, TH. (1903). Die Cirkulations- und Ernährungsverhältnisse des Auges. Leipzig. (15.)
- LEHMANN, G., und MEESMANN, A. (1924). Ueber das Bestehen eines Donnangleichgewichtes zwischen Blut und Kammerwasser bzw. Liquor cerebrospinalis. Pfl. Arch. **205**, 210-232. (12.)
- LERICHE, R., et POLICARD, A. (1920). Etat des capillaires pendant l'excitation du sympathique périartériel chez l'homme. C. R. Soc. Biol. **20**, November, 1920. (5.)
- LEWIS, J. H. (1921). The rate and route of absorption of subcutaneously injected serum in relation to the occurrence of sudden death after injection of antitoxic horse serum. Journ. Amer. Med. Assoc. **76**, 1342. (12, 15.)
- LEWIS, TH. (1923). The force exerted by contracted capillaries. Proc. Physiol Soc. Journ. Physiol. **58**. (8.)
- LEWIS, TH. (1924). The force exerted by the minute vessels of the skin in contracting. Heart, **11**, 109-117. (8.)
- LEWIS, TH. (1924). Vascular reactions of the skin to injury. I. Reaction to stroking. Heart, **11**, 119-139. (10.)
- LEWIS, TH. (1924). Studies of capillary pulsation, with special reference to vasodilatation in aortic regurgitation and including observations on the effects of heating the human skin. Heart, **11**, 151-193. (1.)
- LEWIS, TH. (1926). Observations upon the regulation of blood-flow through the capillaries of the human skin. Heart, **13**, 1-25. (6, 11.)
- LEWIS, TH. (1926). Vascular reactions of the skin to injury. IV. An irresponsive condition of the vessels with special reference to the pathology of telangiectases and allied conditions. Heart, **13**, 153-191. (8.)
- LEWIS, TH. (1927). The blood vessels of the human skin and their responses.
- LEWIS, TH. (1927). The active relaxation of capillaries and venules in the reflex flare. Proc. Physiol. Soc. Journ. Physiol. **64**. (13, A.)

- LEWIS, TH., and GRANT, R. T. (1924). Vascular reactions of the skin to injury. II. Liberation of a histamine-like substance in injured skin.—Observations upon nervous control of certain skin reactions. *Heart*, **11**, 209-265. (6, 10, A.)
- LEWIS, TH., and GRANT (1925). Observations upon reactive hyperaemia in man. *Heart*, **12**, 73-120. (6, 9, 10.)
- LEWIS, TH., and GRANT. Vascular reactions of the skin to injury. VII. Notes on the anaphylactic skin reaction. *Heart*, **13**, 219-225. (10.)
- LEWIS, GRANT, and HARRIS (1927). Observations relating to the influence of the cutaneous nerves on various reactions of the cutaneous vessels. *Heart*, **14**, 1-17. (6.)
- LEWIS, TH., GRANT, and MARVIN (1927). Vascular reactions of the skin to injury. X. The intervention of a chemical stimulus illustrated especially by the flare. The response to faradism. *Heart*, **14**, 139-160. (6, 10.)
- LEWIS and HARMER (1927). Further evidence of the release of a histamine-like substance from the injured skin. *Heart*, **14**, 19-26. (10.)
- LEWIS, TH., and HAYNAL, I. (1928). Observations relating to the tone of the minute vessels of the human skin; with remarks upon and illustrations of measurement of pressure within these vessels. *Heart*, **14**, 177-194. (A.)
- LEWIS, TH., and LOVE (1926). Vascular reactions of the skin to injury. III. Some effects of freezing, of cooling and of warming. *Heart*, **13**, 27-60. (10, 11.)
- LEWIS and MARVIN (1926). Herpes zoster and antidromic impulses. *Proc. Physiol. Soc. Journ. Physiol.* **62**. (5, 9.)
- LEWIS and MARVIN (1927). Observations relating to vasodilatation arising from antidromic impulses, to herpes zoster and trophic effects. *Heart*, **14**, 27-47. (5, 9.)
- LEWIS, TH., and MARVIN (1927). Observations upon a pilomotor reaction in response to faradism. *Journ. Physiol.* **64**, 87-106. (6.)
- LEWIS, TH., and ZOTTERMAN, Y. (1926). Vascular reactions of the skin to injury. V. Annular edema of the skin in a case of infective endocarditis. *Heart*, **13**, 193-201. (14.)
- LEWIS, TH., and ZOTTERMAN (1926). Vascular reactions of the skin to injury. VI. Some effects of ultra-violet light. *Heart*, **13**, 203-217. (10.)
- LEWIS, TH., and ZOTTERMAN (1927). Vascular reactions of the skin to injury. VIII. The resistance of the human skin to constant currents, in relation to injury and vascular response. *Journ. Physiol.* **62**, 280-288. (10.)
- LIEBEN (1910). Fortbewegung d. Lymphe in den Lymphgefäßen. *Zentralbl. f. Physiol.* **1910**, 1164. (4.)
- LIEBERMANN, P. v. (1921). Polare Erregung und Hemmung an Arterien. *Pfl. Arch.* **192**, 130-134. (8.)
- LISTER (1858). The early stages of inflammation. *Phil. Trans.* **148**, 645. (3.)

- LOEB, J. (1922). Proteins and the theory of colloidal behavior. McGraw-Hill, New York. (12.)
- LOMBARD, W. P. (1912). The blood pressure in the arterioles, capillaries, and small veins of the human skin. *Amer. Journ. Physiol.* **29**, 335. (4.)
- LOVÉN, CHR. (1866). Ueber die Erweiterung von Arterien in Folge einer Nervenirregung. *Verhandl. d. sächs. Ges. d. Wis. M. ph. Cl.* **1866**, 85. (7.)
- LUNGWITZ (1910). *Der Fuss des Pferdes*, p. 157. (13.)
- MAGNUS, G. (1926). Exp. Untersuchunge z. Frage d. Gefässinnervierung. *Arch. f. klin. Chir.* **143**, 547-581. (5.)
- MAGNUS, R. (1899). Ueber die Entstehung der Hautödeme bei experimenteller hydrämischer Plethora. *Arch. f. exper. Path. u. Pharm.* **42**, 250. (13, 14.)
- MAJOR and STEPHENSON (1924). *Journ. Amer. Med. Ass.* **83**. Johns Hopkins Hosp. Bull. **1924**. (8.)
- MALL, J. P. (1887). Die Blut- und Lymphwege im Dünndarm des Hundes. *Abh. d. sächs. Ges. d. Wiss. M. ph. Cl.* **14**, 153. (2, 15.)
- MARCHAND, F. (1923). Ueber die Kontraktilität der Kapillaren und die Adventitialzellen. *Münch. Med. Woch.* **70**, 385-397. (4.)
- MARRACK, J., and HEWITT, L. F. (1927). The effect of hydrogen ion concentration and protein concentration on the osmotic pressure of serum proteins. *Biochem. Journ.* **21**, 1129-1140. (12.)
- MAUTNER (1924). Ueber die pharmakologische Beeinflussung der Leber. *Klin. Woch.* **3**, 2321-2325, 2369-2372. (11.)
- MAUTNER and PICK (1915). Ueber die durch "Schockgifte" erzeugten Zirkulationsstörungen. *Münch. Med. Woch.* **62**, 1141. (11.)
- MAXIMOW, A. (1926). Ueber undifferenzierte Blutzellen und mesenchymale Keimlager im erwachsenen Organismus. *Klin. Woch.* **5**, No. 47. (4.)
- MAYER, S. (1902). Die Muskularisierung der capillaren Blutgefäße. *Anat. Anz.* **21**, 442. (4.)
- MAYRS, E. B. (1926). The functional pathology of nephritis. *Quart. Journ. Med.* **19**, 273-297. (12.)
- MEDICAL RESEARCH COMMITTEE (1919). Wound-Shock and Hemorrhage. *Spec. Rep. Series*, No. **25**. (9, 10, 15.)
- MEEK, W. J., and EYSTER, J. A. E. (1921). Reactions to hemorrhage. *Amer. Journ. Physiol.* **56**, 1-15. (11.)
- MEEK, W. J., and EYSTER, J. A. E. (1922). The effect of plethora and variations in venous pressure on diastolic size and output of the heart. *Amer. Journ. Physiol.* **61**, 186-201. (11.)
- MENDE (1919). Ueber Hyperämie und Oedem bei der Hemmung des Rückflusses des venösen Blutes durch die Staubinde. *Deutsche Zeitschr. f. Chirurgie*, **150**, 379. (15.)
- MENDEL (1922). Edema cutis proprium. *Klin. Woch.* **1**, 1502. (15.)
- MÜLLER, JOH. (1834). *Handbuch d. Physiol. des Menschen*. **1**. (3.)
- MÜLLER, L. R. (1913). Studien über den Dermoglyphismus und dessen



- diagnostische Bedeutung. Deutsch. Zeitsch. f. Nervenheilk, **47-48**, 413-435. (6, 7.)
- MÜLLER, O. (1922). Die Capillaren der menschlichen Körperoberfläche in gesunden und kranken Tagen. Enke, Stuttgart. (1, 6, 7, 11.)
- MÖLLENDORF, W. v. (1927). Einige Beobachtungen über den Aufbau des Nierenglomerulus. Zeitsch. f. Zellforsch. u. mikr. Anat. **6**, 441-450. (4.)
- NATUS, M. (1910). Beiträge zur Lehre von der Stase. Arch. f. path. Anat. u. Physiol. **199**, 1. (8.)
- NEERGAARD, K. v. (1927). Zur Frage des Druckes im Pleuraspalt. Beiträge z. Klin. d. Tuberk. **65**, 476-485. (15.)
- NEUBURGER, J. (1927). Die Stigmata-Frage. Deutsch. Med. Woch. **53**, 193. (5.)
- NI, T. G. (1922). Active response of capillaries. Amer. Journ. Physiol. **62**, 282, **63**, 425. (8.)
- NITSCHKE (1928). Ueber die Bedeutung d. Membran bei d. Messung des osmotischen Druckes der Plasmaeiweisskörper. Zeitsch. g. exp. Med. **59**, 298-302. (12.)
- NORDMANN, M. (1925). Studien am Fettgewebe, insbesondere dem des lebenden Säugetieres. Zeitsch. exp. Med. **48**, 84-110. (5.)
- OERSKOV (1925). Observations sur la propriété phagocytaire des endothéliums capillaires. B. C. R. Soc. Biol. **93**, 959. (4.)
- OINUMA, S. (1924). Variation of capillary diameter and antidromic action in the frog. Journ. of Physiol. **58**, 318-326. (3.)
- OLIVECRONA, H. (1922). An experimental study of the circulatory failure in peritonitis. Acta Chir. Scand. **54**. (15.)
- PARKER (1923). Rouget cells on blood-vessels of invertebrates. Anat. Record, **26**, 303. (4.)
- PARRISIUS, W. (1921). Capillarstudien bei Vasoneurosen. Deutsche Zeitsch. f. Nervenheilk, **72**, 310. (11.)
- PFUHL, W. (1926). Experimentelle Untersuchungen über die Kupffer-schen Sternzellen der Leber. I. Die verschiedenen Formen der Sternzellen, ihre Lage in den Lebercapillaren und ihre allgemeine Biologie. Zeitsch. Anat. u. Entwickl. **81**, 90-114. (4.)
- PHILIP, A. W. (1804). A treatise on febrile diseases. (Stegemann quotes a German translation from the third edition. Leipzig, 1804.) (3.)
- PHILIP, A. W. (1817). The laws of the vital functions. Third edition, London, 1826. (First ed., 1817.) (3.)
- POULSSON, L. (1926). Ueber die exsudationshemmende Wirkung des Pituitrins. Arch. f. exp. Path. u. Pharm. **120**, 120. (9, 15.)
- POULSSON, L. T. (1926). Observations experimentales sur l'action de la pituitrine et de l'histamine sur la pression arterielle. Physiol. Papers. Dedicated to Prof. A. Krogh, Levin & Munksgaard, Copenhagen, 1926. 232-247. (9.)
- RAJKA, E., und FÜRTH (1924). Ueber die Genese der reflektorischen Umgebungshyperämie unter physiologischen und pathologischen Verhältnissen. Deutsche Med. Woch. **1924**, 1-5. (6.)
- RAJKA und WESSELY (1923). Zeitsch. f. d. ges. exp. Med. **57**, 171. (4.)

- RANSON and WIGHTMAN (1922). The vasodilator fibers of the dorsal roots. *Amer. Journ. Physiol.* **62**, 392. (5.)
- RECKLINGHAUSEN, H. v. (1906). Unblutige Blutdruckmessung. *Arch. f. exper. Path. u. Pharm.* **55**, 376. (13.)
- REHBERG, P. B. (1926). Studies on kidney function. *Biochem. Journ.* **20**, 447-482. (2, 15.)
- REHBERG, P. B., and CARRIER, E. B. (1922). Concerning the reaction of the human skin capillaries to venous blood. *Skand. Arch. Physiol.* **42**, 250-265. (6.)
- RETZIUS, G. (1891). Ueber Nervenendigungen an den Parapodienborsten und über die Muskelzellen der Gefässwände bei den polychaeten Annulaten. *Verh. Biol. Ver. Stockholm*, **3**, 85-89. (4.)
- RETZIUS, G. (1905). Ueber Muskelzellen an den Blutgefässen der Polychaeten. *Biol. Untersuch. Neue Folge*, **12**, 75-78. (4.)
- RIBBERT, H. (1909). Wesen der Krankheiten. (Quoted from *Handwörterbuch d. Naturwiss.* **VII**, 543.) (15.)
- RICH, A. R. (1921). Condition of the capillaries in histamine shock. *Journ. exper. Med.* **33**, 287. (9, 11, A.)
- RICHARDS, A. N. (1922). Kidney function. *Amer. Journ. Med. Sci.* **163**, 1. (11.)
- RICHARDS and SCHMIDT (1925). Glomerular circulation in the frog's kidney. *Amer. Journ. Physiol.* **71**, 178-208. (4, 11.)
- RICKER, S., und REGENDANZ (1921). Beiträge zur Kenntniss der örtlichen Kreislaufstörungen. *Virchow's Arch.* **231**, 1. (8, 11.)
- ROHRER, F. (1916). Bestimmung des Mischungsverhältnisses von Albumin und Globulin im Blutserum. *Deutsche Arch. f. kl. Med.* **121**, 221-240. (12.)
- ROSENOW, G. (1916). Der Einfluss parenteraler Calciumzufuhr auf die Durchlässigkeit der Gefässwand. *Zeitsch. f. d. ges. exp. Med.* **4**, 427. (14.)
- ROSENTHAL, W. (1921). Phagocytose durch Endothelzellen. *Zeitsch. f. Immunitätsforsch.* **31**, 372-385. (4.)
- ROUGET, CH. (1873). Mémoire sur le développement de la tunique contractile des vaisseaux. *C. R.* **79**, 559. (4.)
- ROUGET, CH. (1879). Sur la contractilité des capillaires sanguins. *C. R.* **88**, 916. (4.)
- ROUS, DRURY, and BEATTY (1927). Relative reaction within living mammalian tissue. *Journ. exp. Med.* **45**. (8.)
- ROY, CH., and GRAHAM BROWN (1879). The blood pressure and its variations in the arterioles, capillaries, and veins. *Journ. Physiol.* **2**, 323. (3.)
- RUHMANN (1927). Ueber viscerele Reflexe auf lokale thermische Hautreize. III. *Zeitsch. f. d. ges. exp. Med.* **57**, 768-797. (7.)
- RUNGE und KESSLER (1925). Beiträge z. Physiologie des Wasser-stoffwechsels in der Schwangerschaft. *Arch. f. Gynäkologie*, **126**, 45. (12.)
- RUSZNYAK, ST. (1924). Untersuchungen über die Entstehung des Ödems bei Nierenkranken. *Zeitschr. g. exp. Med.* **41**, 532. (12.)

- SABIN, F. R. (1920). Studies on the origin of blood vessels and of red blood-corpuscles. Carnegie Publ. No. 272, 1920. Contr. to Embryology, **9**, 213-262. (4.)
- SABIN, F. R. (1922). Direct growth of veins by sprouting. Contr. to Embryology, **14**, 1-10. (4.)
- SABIN and DOAN (1926). Journ. exp. Med. **43**, 823. (4.)
- SACKS, B. (1924). Observations upon the vascular reactions in man in response to infundin, with special reference to the behaviour of the capillaries and venules. Heart, **11**, 353-370. (9.)
- SANDOR, G. (1926). Vergleichende Untersuchungen an Froschgefäßen mit besonderer Berücksichtigung des Gehirns. Pfl. Arch. **213**, 492-510. (8.)
- SCHADE, H. (1927). Ueber Quellungsphysiologie und Ödementstehung. Ergb. der inn. Med. u. Kinderheilk. **32**, 425-463. (12, 13.)
- SCHADE, H., und CLAUSSEN, F. (1924). Der onkotische Druck des Blutplasmas und die Entstehung der renal bedingten Ödeme. Zeitsch. klin. Med. **100**, 363-410. (12, 15.)
- SCHAFER, E. (1902). On the existence within the liver cells of channels which can be directly injected from the blood vessels. Proc. Roy. Soc. Edinburgh, **24**, 65. (4.)
- SCHAFER, J. (1920). Histologie und Histogenese. (4.)
- SCHALY, G. A. (1926). Over het Voorkomen van de Cellen van Rouget op den Wand van de Capillairen in het Oog van den Mensch. Dissertation, Groningen, 1-72. (4.)
- SCHKLAREWSKY, A. (1868). Ueber das Blut und die Suspensions-flüssigkeiten. Pfl. Arch. **1**, 603. (1.)
- SCHKLAREWSKY, A. (1868). Zur Extravasation der weissen Blutkörperchen. Pfl. Arch. **1**, 657. (1.)
- SCHULEMANN, W. (1917). Vitale Färbungen mit sauren Farbstoffen. Biochem. Zeitschr. **80**, 1. (12.)
- SCHUR, H. (1920). Haut und Hautcapillaren im mikro-episkopischen Bilde, Zeitschr. f. angew. Anat. u. Konstitutionslehre, **5**, 193. (4.)
- SCOTT, F. H. (1916). The mechanism of fluid absorption from tissue spaces. Journ. Physiol. **50**, 157. (13.)
- SEIDEL, E. (1921). Exp. Untersuch. über intraokulare Saftströmung. IX. Ueber den Abfluss von Kammerwasser. Arch. f. Ophthal. **104**, 357. (15.)
- SEIDEL, E. (1922). Weitere exp. Untersuch. über die Quelle und der Verlauf der intraokularen Saftströmung. XVI. Mitteilung. Arch. f. Ophthal. **108**, 285. (15.)
- SEIDEL, E. (1927). Methoden z. Untersuchung des intraokularen Flüssigkeitswechsels. Abderhald. Handb. d. biol. Arbeitsmeth. Abt. V. Teil **6**, 1064-1078. (15.)
- SERR, H. (1924). Blutbeschaffenheit u. Glaukom. Arch. f. Ophthalm. **114**, 393. (12.)
- SHEARD, CH. (1924). Instantaneous photomicrography of the skin capillaries in the living human body. Science, **60**, 409-410. (4.)
- SHERINGTON, C. S. (1920). The integrative action of the nervous system. Sixth printing, Yale University Press. (4, 6.)

- SIPERSTEIN, D. M., and SANSBY, J. M. (1923). Intraperitoneal transfusion with citrated blood. *Amer. Journ. of Diseases of Children*, **25**, 107-129. (15.)
- SLONIMSKI, P. (1927). Ueber die Darstellung winziger Blutgefäße mittels der Benzidinprobe. *Zeitsch. f. wiss. Mikrosk.* **44**, 1-8. (4.)
- SMITH, H. P., ARNOLD, H. R., and WHIPPLE, S. H. (1921). Blood volume studies. VII. *Amer. Journ. Physiol.* **56**, 336. (1.)
- SPALTEHOLZ, W. (1888). Die Vertheilung der Blutgefäße im Muskel. *Abhd. d. sächs. Ges. d. Wiss. M.-Ph. Cl.* **14**, 509. (2.)
- SPALTEHOLZ, W. (1893). Die Verteilung der Blutgefäße in der Haut. *Arch. f. Anat. (u. Physiol.)* **1893**, 1. (2, 4.)
- SPALTEHOLZ, W. (1927). Blutgefäße der Haut. *Handb. der Haut- und Geschlechtskrankh.* **1**, 379-433. (4.)
- SPERANSKAJA-STEPANOWA, E. (1925). Ueber die Kreuzung der die Hautdrüsen und die Blutgefäße der hinteren Extremitäten versorgenden sympathischen Grenzstrangfasern beim Frosch. *Pfl. Arch.* **210**, 627. (7.)
- SPERANSKAJA-STEPANOWA, E. (1925). Postganglionäre sympathische vasoconstrictorische und vasodilatatorische Axonreflexe. *Pfl. Arch.* **210**, 633-640. (7.)
- STARLING, E. H. (1894). The influence of mechanical factors on lymph production. *Journ. Physiol.* **16**, 224. (13.)
- STARLING, E. H. (1896). On the absorption of fluid from the connective tissue spaces. *Journ. Physiol.* **19**, 312-327. (12.)
- STARLING, E. H. (1899). The glomerular functions of the kidney. *Journ. Physiol.* **24**, 317-330. (15.)
- STARR, J., JR. (1925). Production of albuminuria by renal vasoconstriction. *Amer. Journ.* **72**, 184. (15.)
- STARR, J., JR. (1926). The production of albuminuria by renal vasoconstriction in animals and man. *Journ. Exp. Med.* **43**, 31-52. (15.)
- STEGEMANN, H. (1927). Vergessene Capillarbeobachtungen. *Klin. Woch.* **6**, 412-416. (3.)
- STEINACH, E., und KAHN, R. H. (1903). Echte Contractilität und motorische Innervation der Blutcapillaren. *Pfl. Arch.* **97**, 105. (3.)
- STERNBERG, H. (1927). Ueber die Blutverteilung resp. Capillarinjektion in der Schleimhaut der Luftwege und ihre physiologische und pathologische Bedeutung. *Zeitsch. f. Hals- Nasen- und Ohrenheilk.* **18**, 593, 598. (11.)
- STEWART, G. N. (1895). *Manual of Physiology*. London, 1895, p. 59. (4.)
- STEWART, G. N. (1911). Studies on the circulation in man. I. Heart, **3**, 33. (7.)
- STILWELL, F. (1926). On the phagocytic capacity of the blood vessel endothelium of the frog's tongue and its presumed transformation into wandering cells. *Folia hæmatolog.* **33**, 81-94. (4.)
- STOEL, G. (1925). Ueber die Blutversorgung v. weissen u. roten Kaninchenmuskeln. *Zeitsch. f. Zellforsch. u. mikr. Anat.* **3**, 91-98. (2.)
- STRICKER, S. (1865). Studien über Bau u. Leben der capillaren Blutgefäße. *Sitzungsber. d. Wiener Akad. d. Wiss. M.-N. Kl.* **52**, 2 Abt. 379. (3.)

- STRICKER, S. (1879). Untersuchungen über die Contractilität der Capillaren. Sitzungsber. d. Wiener. Akad. d. Wiss. M-N. Kl. **74**, 3 Abt. 313. (4.)
- STÖHR, P., JR. (1926). Mikroskopischer Beitrag z. Innervation der Blutcapillaren beim Menschen. Zeitsch. f. Zellforsch. **3**, 431-447. (5.)
- STÖHR, PH., JR. (1927). Anatomische Beobachtungen und Bemerkungen über den Aufbau des sympathischen Nervensystems. Klin. Woch. **6**, No. 21. (5.)
- STÖHR, PH., JR. (1927). Beobachtungen und Bemerkungen über den Aufbau des sympathischen Grenzstranges. Zeitsch. f. Zellforsch. u. mikr. Anat. **5**, 117-149. (5.)
- SØRENSEN, S. P. L. (1915-1917). Proteinstudier. Medd. fra Carlsberg Lab. **12**. (12.)
- TANNENBERG, J. (1925). Experimentelle Untersuchungen über lokale Kreislaufstörungen. III. Die Stase, zugleich Untersuchungen über die Entstehungsbedingungen eines Kollateralkreislaufes. Frankf. Zeitsch. f. Path. **31**, 285-350. (1.)
- TANNENBERG, J. (1925). Experimentelle Untersuchungen über lokale Kreislaufstörungen. IV. Die Leukozytenauswanderung und die Diapedese der roten Blutkörperchen. Frankf. Zeitsch. f. Path. **31**, 351-384. (1.)
- TANNENBERG, J. (1925). Ueber die Capillartätigkeit. Zeitsch. f. allg. Path. u. path. Anat. **36**, Erg. heft. 374. (4.)
- TANNENBERG, J. (1926). Bau und Funktion der Blutkapillaren. Deutsche Med. Woch. **1926**, No. 10. (4.)
- THOMPSON, W. O., THOMPSON, P. K., and DAYLEY, M. E. (1928). The effect of posture upon the composition and volume of the blood in man. Proc. Nat. Acad. Sc. U.S.A. **14**, 94-99. (15.)
- TOYAMA, K. (1925). Exp. Forschung über die Lungenkapillaren. Zeitsch. f. d. ges. exp. Med. **46**, 168. (11.)
- TROTTER, W., and DAVIES, H. M. (1909). Experimental studies in the innervation of the skin. Journ. Physiol. **38**, 134-246. (6.)
- TÖRÖK, L. (1928). I. Zirkulationsstörungen der Haut. II. Angioneurosen. III. Urticaria. IV. Urticaria pigmentosa. V. Nachtrag zum Kapitel Entzündung. Handb. der Haut- und Geschlechtskrankh. **6**, 1-679. (10, 15.)
- TÖRÖK, LEHNER, und URBAN (1925). Ueber Veränderungen der Reaktion der Haut nach wiederholten Einwirkungen auf dieselbe Hautstelle. Krankheitsforsch. **1**, 371-406. (6, 11.)
- TÖRÖK, L., und RAJKA, E. (1924). Beitrag zur Pathogenese der Hyperämie und des Ödems bei der Urticaria und der akuten Entzündung der Haut. Arch. f. Dermat. u. Syphilis. **147**, 559-580. (6, 15.)
- TÖRÖK, L., und RAJKA, E. (1925). Experimentelle Untersuchungen über die Entstehung des hyperämischen Entzündungshofes. Wien. Med. Woch. **1925**, No. 6. (6.)
- UEKI, R. (1924). Versuche mit gummiarabikumhaltigen Blutersatzflüssigkeiten. Arch. f. exper. Path. u. Pharm. **104**, 239-249. (15.)
- VERNEY, E. (1926). The osmotic pressure of the proteins of human serum and plasma. Journ. Physiol. **61**, 319-328. (12.)



- VERZAR, P. (1912). The influence of lack of oxygen on tissue respiration. *Journ. Physiol.* **45**, 39. (12.)
- VIMTRUP, B.J. (1922). Beiträge zur Anatomie der Capillaren. I. Ueber contractile Elemente in der Gefäßwand der Blutcapillaren. *Zeitsch. f. d. ges. Anat.* **65**, 150. (4, A.)
- VIMTRUP, B.J. (1923). Beiträge zur Anatomie der Capillaren. II. Weitere Untersuchungen über contractile Elemente in der Gefäßwand der Blutcapillaren. *Zeitsch. f. d. ges. Anat.* **68**, 469-482. (4.)
- VIMTRUP, B.J. (1926). Ueber die Malpighi'schen Körperchen der menschlichen Niere. *Physiol. Papers. Dedicated to Prof. Aug. Krogh, Levin & Munksgaard, Copenhagen, 1926.* 357-375. (4.)
- VIMTRUP, B.J. (1928). Undersøgelse over Antal, Form, Bygning og Overflade af Glomeruli i Nyren hos Menneske og nogle Pattedyr. *Danske Vid. Selsk. Biol. Medd.* **7**, 1-36. (2, 14.)
- VIMTRUP, B.J. (1928). On the number, shape, structure, and surface area of the glomeruli in the kidneys of man and mammals. *Amer. Journ. Anat.* **41**, 123-151. (2, 14.)
- VOLHARD, A. (1917). Die doppelseitigen hämatogenen Nierenerkrankungen (Brightsche Krankheit). *Handb. d. inn. Med.* III 2, 1148-1722. (12, 15.)
- VOLLMER und LEE (1927). Z. Physiologie des Schreiwinsens der Säuglinge. *Klin. Woch.* **6**, 1990. (15.)
- VOLTERRA, M. (1925). Einige neue Befunde über die Struktur der Kapillaren und ihre Beziehungen zur sogenannten Kontraktilität derselben. *Zentralbl. f. inn. Med.* **1925**, 876-881. (4.)
- VOLTERRA, M. (1925). Considerazioni sulla struttura dei capillari sanguigni e su una categoria di cellule a carattere emoistioblastico in rapporto all'anatomia-patologica e alla fisiopatologia.—La contrattilità capillare. *Lo Sperimentale. Arch. di Biol. norm. e patol.* **79**, 1-24. (4.)
- VOLTERRA, M. (1925). Sulla struttura dei capillari sanguigni e l'anatomia del sistema reticolo-endoteliale. *Monitore Zoo. Italiano*, **34**, 49-58. (4.)
- VONWILLER, P. (1924). Neue Mikroskopiermethode für Beobachtung lebender Organismen. *Zeitsch. wiss. Mikro.* **41**, 190. (4.)
- WALPOLE, G. S. (1915). Notes on collodion membranes for ultrafiltration and pressure dialysis. *Biochem. Journ.* **9**, 284. (4.)
- WEARN, J. T. (1926). *Proc. Soc. Exp. Biol. and Med.* **23**, 707-708. (2.)
- WEARN (1928). The extent of the capillary bed of the heart. *Journ. Exp. Med.* **47**, 273, 291. (1, 2.)
- WEARN (1928). The rôle of the Thebesian vessels in the circulation of the heart. *Journ. Exp. Med.* **47**, 293-316. (4.)
- WEARN, BARR, and GERMAN (1926). Behavior of arterioles and capillaries of the lung. *Proc. Soc. exp. Biol. and Med.* **24**, 114-115. (11.)
- WEDEMEYER, C. (1828). Untersuchungen über den Kreislauf des Blutes. Hannover, 1828. (3.)
- WEIL (1924). Ueber die Nachwirkung thermischer Reize. *Zeitsch. f. klin. Med.* **101**, 195-204. (8, 11.)

- WEISS, E. (1916). Beobachtung und mikrophot. Darstellung der Hautcapillaren am lebenden Menschen. Deutsch. Arch. f. klin. Med. **119**, 1. (4.)
- WERNÖE, TH. B. (1920). Aesthesioscopia abdominalis (Danish) Ugeskrift for Læger. **82**, 1415. (7.)
- WERNÖE, TH. B. (1925). Viscero-cutane Reflexe. Pfl. Arch. **210**, 1-34. (7.)
- WERNÖE, TH. B. (1926). Ueber den Verlauf und die Verteilung präganglionärer sympathischer Bahnen bei Fischen. Physiol. Papers. Dedicated to Prof. A. Krogh, Levin & Munksgaard, Copenhagen, 290-307. (7.)
- WERNÖE, TH. B. (1927). Le reflexe naso-oculaire vasodilatatoire et sa valeur diagnostique. Acta psych. et neur. **2**, 385, 398. (7.)
- WETZEL, N. C., and ZOTTERMAN, Y. (1926). On differences in the vascular colouration of various regions of the normal human skin. Heart, **13**, 358-370. (2.)
- WHITE (1924). On glomerular filtration. Amer. Journ. **68**, 523-530. (12.)
- WHITE and ERLANGER (1920). Blood analysis following acacia-glucose injections. Amer. Journ. Physiol. **54**, 1-29. (13.)
- WIEDHOFF (1923). Wirkung der periarteriellen Sympathectomie. Bruns Beitr. z. klin. Chir. **130**, 399. (5.)
- WITH, C. (1920). Studier over Lysets Virkning ved Vitiligo (Danish). Hospitalstid. **1920**. (10.)
- WOLF, E. P. (1921). Experimental studies on inflammation. I. The influence of chemicals upon the chemotaxis of leucocytes in vitro. Journ. exp. Med. **34**, 375-396. (15.)
- WOLF, E. P. (1923). Experimental studies on inflammation. II. Experimental chemical inflammation in vivo. Journ. exper. Med. **37**, 511-524. (15.)
- WOLF, E. P. (1924). Local changes of colour in the skin deprived of its normal blood supply. Heart, **11**, 327-335. (11.)
- WOODLAND, W. (1911). On the structure and function of the gas glands and retia mirabilia . . . of some teleostean fishes. Proc. Zool. Soc. London, **1911**, 183-249. (2.)
- WOOLLARD, H. H. (1926). The innervation of blood vessels. Heart, **13**, 319-336. (5.)
- WORM-MÜLLER, J. (1873). Die Abhängigkeit des arteriellen Blutdruckes von der Blutmenge. Verhandl. d. sächs. Ges. d. Wiss. M-Ph. Kl. **1873**, 172. (11.)
- ZAK, E. (1922). Ueber vasomotorische Zonen bei Erkrankungen der Aorta. Wien. Arch. f. inn. Med. **4**, 209-234. (7.)
- ZIMMERMANN, K. W. (1923). Die feinere Bau der Blutcapillaren. Zeitsch. f. Anat. u. Entwickl. **68**, 29-109. (4.)
- ZONDEK, H. (1921). Die Bedeutung kolloidaler Nahrösungen für die Funktion des normalen, erschöpften und vergifteten Herzens. Biochem. Zeitsch. **116**, 246. (15.)
- ZSIGMONDY, R., und BACHMANN (1918). Ueber neue Filter. Zeitsch. f. anorganische Chemie, **103**, 119-128. (4.)



## INDEX

- Abrine, reactions in conjunctiva, 222.
- Absorption, from abdominal cavity, 366; from pleural cavity, 340, 365; of fluid from thoracic cavity, 340; of edema after diuretic drugs, 366; of edema through lymph channels, 359, 365; of substances from intestine, 341; of water into the blood, 279, 365.
- Acetyl choline, action on circulation, 57; compared with histamine, 202; in perfusion fluids, 188.
- Adrenaline, action on blood vessels, 175; action on circulation, 57; action on chromatophores in fishes, 146; action on human skin capillaries, 86, 179; action on Rouget cells, 179; as a circulatory hormone, 194; in perfusion fluids, 189; reversal of reactions to—after abrine, 223; unresponsiveness of vessels to, 175, 177, 178, 180.
- Adventitial cells, 82; from connective tissue, 84; in sprouting vessels, 94.
- Agglutination (*see* red corpuscles), 13.
- Angioblasts, 93.
- Anoxemia (*see* oxygen).
- "Antidromic" innervation (*see also* dilator), 116, 119, 121; in local reflex, 124.
- Aqueous humor, filtration of—from anterior chamber of eye, 345.
- Arteries (*see also* arterioles), contraction on mechanical stimulation, 20, 143; relaxation of—during occlusion, 225.
- Arterioles (*see also* arteries), human skin, 35, 97, 130; increase in tone of—as a response to pressure, 231; in microscopic picture, 4; in temperature regulation, 154; in regulating blood flow, 4, 294; relaxation of—during occlusion, 231.
- Arteriomotor, control of circulation, 248, 366.
- Arterio-venous anastomoses, 100; in the heart, 106; possible occurrence in arctic animals, 105; rôle in heat regulation, 103.
- Ascites, portal pressure causing, 265.
- Axial flow, of corpuscles (*see* blood flow), 5.
- Axon reflex, affected by nerve sections, 127, 128; in conjunctiva, 124, 139; in ears of rabbit, 140, 150; in erythema, 123; in internal organs, 140; in sympathetic system, 139, 143, 158; long path, 144; preganglionic, 144; spreading of—in human skin, 129.
- Bacterial poisons, slow reactions to, 223.
- Bier's white spots, 245.
- Blister, as a reaction to light, 218; by plasma filtration, 316; caused by mustard gas, 222; formation of, 353.
- Blood flow, axial flow, 5; cinema pictures of, 10, 14, 19; granular, 13; in flare, 130; in lung, 9; in reactive hyperemia, 225, 228; irregularities of, 12; measured by temperature, 157; observation of—in transparent tissue, 3, 369; plasma skimming, 7, 20; plasma zone, 5, 16; rate of—in small vessels, 3, 11; stoppage of, 13; time of passage for 1 mm.<sup>3</sup>, 104.
- Blood pressure, determination of

- in capillaries, 385; determination of—in veins, 302; difference between arterial and venous capillaries, 293; effect on capillary diameter, 68, 126, 141; in capillaries, 295; in glomeruli, 349; in human venules in flare, 131; in lower part of body, 305; in mesentery vessels of frog, 299, 318; in reflex erythema, 298; in shock, 354, 357; in veins, 298, 301; in veins, causing edema, 359.
- Blood volume regulation, 256, 264.
- Blushing, 154.
- “Blutgefühl,” 49; in reactive hyperemia, 230.
- Caffeine, capillary reactions to, 174.
- Calcium, action on capillary permeability, 336; in gum, 367; action in inflammation, 354.
- Cantharidine, chemotactic action of, 351.
- Capillariomotor, control of circulation, 248, 318.
- Capillary poisons, 197.
- Capillary wall (*see also* endothelium, Rouget cells), development of, 93; diapedesis through, 15, 16; invisibility of—in reflected light, 19; methods for histological study of, 376; possibility of openings in, 317; sprouting of, 21; stretching of—increasing permeability, 317, 335; structure of, 70; varicose dilatations, 16.
- Carbonic acid, capillary reactions to, 171; diffusion of—in tissues, 273.
- Cardiac insufficiency, causing edema, 361.
- Chemical stimulation of capillaries, in human skin, 209; in the frog's tongue and web, 125, 126.
- Chemotaxis (*see also* white corpuscles), 21, 351.
- Chromatophores, reactions to pituitary, 182; reflex reactions of, 146; sympathetic innervation of—in fishes, 146.
- Chyle, absorption of—into blood, 341; concentration of substances in, 343.
- Cinematography, methods, 372; of blood flow, 11, 14, 19; of vascular reactions, 123, 177.
- Closed capillaries, 251; alternation of, 253; in muscles, 61, 253, 269.
- Cold, effect of—on Bier's spots, 247; effect of—in wound shock, 355; local vascular reactions to, 156, 167, 180; reflex reaction to, 151.
- Colloid, impermeability of capillary wall to, 279.
- Colloid osmotic pressure, 283; equilibrium with blood pressure, 293, 299; fractional, 286; in renal disease, 265; methods for measuring, 383; of albumins and globulins, 284; of blood in different animals, 288; of blood proteins, 285, 292; of fibrinogen, 287; of frog's blood, 288, 301; of gum, 286, 367; of human blood, 289; related to capillary pressure, 311, 318; relation to concentration, 285.
- Color adaptation, by capillary reactions in Fundulus, 260.
- “Conductive pattern” in peripheral nerves, 142.
- Contractility of capillaries (*see also* Rouget cells), 47; history of, 48; independently of Rouget cells, 84, 86, 91; in glomeruli, 96.
- Crystalloids, attraction of water by, 312; exchange of—through capillary wall, 274; rate of exchange of—independent of water movement, 327.
- Denervation, effect, and reactive hyperemia, 229; effect on in-



- flammation and urticaria, 353;  
 effect on light reaction, 217; ef-  
 fect on temperature, 60; white  
 reaction after, 164.
- Depressor (*see* pressor).
- "Derivating channels" (*see* ar-  
 terio-venous anastomoses).
- Dermographic reactions (*see also*  
 white reaction, flare), 55.
- Diapedesis (*see* red corpuscles),  
 15.
- Diet, influence of—on skin capil-  
 laries, 259.
- Diffusion, flush after freezing, 219;  
 flush in light reactions, 219, 333;  
 methods for measuring gas—,  
 382; of crystalloids through capil-  
 lary wall, 274; of dyes in capil-  
 laries, 278, 327; of H-substance,  
 211; of oxygen from capillaries  
 to tissue, 269; of oxygen through  
 tissue membranes, 268, 382; of  
 substances acting on capillaries,  
 162; of substances from capil-  
 laries, 253; of urea through tis-  
 sues, 274.
- Dilator innervation, by sensory  
 fibers, 118; indirect action of,  
 117; of capillaries, 115.
- Dilator substances (*see also* H-sub-  
 stance), 195; hormone, 207;  
 from albumoses and peptones,  
 241; in muscle, 257; in reactive  
 hyperemia, 232, 238; liberated  
 by heat, 214; liberated by sugar,  
 215; of low diffusibility, 220,  
 233, 237, 238, 240; produced by  
 irradiation, 241.
- Donnan effect, in colloid osmotic  
 pressure, 283.
- Edema, 357; by plasma filtration,  
 315; by filtration of water, 304,  
 359; in lungs, 313; intracellular,  
 358; latent, 331; of liver surface,  
 334; rate of formation of, 360,  
 368.
- Electrical stimulation - (*see also*  
 faradic), 166.
- Emigration (*see* white corpuscles).
- Endothelium, 70; ameboid move-  
 ment, 95; changes in—during  
 contraction, 81; contractility of,  
 84, 86, 91; elasticity, 47; folding  
 of, 81, 86; nuclei of, 70, 81,  
 83, 84, 96; passage of corpuscles  
 through, 15, 17, 18; peristalsis  
 of, 105; phagocytosis by, 95;  
 supposed swelling of, 71, 73;  
 thickness of, 92.
- Erythrocytes (*see* red corpuscles).
- Faradic stimulation, 52; producing  
 flare, 135.
- Filtering capacity, of canal of  
 Schlemm, 345; of frog's capil-  
 laries, 320, 349; of glomeruli,  
 348; of membranes, 287, 292, 384.
- Filtration (*see also* filtering ca-  
 pacity), of aqueous humor, 345;  
 of dye substances, 327; of fluid  
 by congestion, 311; of fluid by  
 dilution of blood, 311; of fluid  
 from arterial capillaries, 287; of  
 fluid from blood in working or-  
 gans, 312; of fluid from capil-  
 laries in the frog, 301, 309; of  
 fluid into thoracic cavity, 340;  
 of glomerular urine, 348; of  
 plasma, 316; edema, 304; rate,  
 measured on frog's capillaries,  
 320; rate of slowest substance,  
 309.
- Flare (*see also* reflex erythema),  
 127; absence of—in light reac-  
 tions, 218; absence of—in mus-  
 tard gas reactions, 222; affected  
 by occlusion, 130, 140, 211; af-  
 fected by reactive hyperemia,  
 136; as a spinal reflex, 128; by  
 faradic stimulation, 135; biolog-  
 ical significance of, 258; exten-  
 sion by steps, 139; in areas with  
 weakened tone, 129; in rabbit's  
 ear, 140; limitation of, 139, 141;

- nerve fibers involved in, 133;  
 produced by injurious stimuli,  
 209; produced directly by nerve  
 stimulation, 240; reflex path in,  
 131; venule pressure in, 298;  
 vessels reacting in, 129, 140.
- Freezing, diffusion flush after, 219;  
 direct action on pain nerves, 241;  
 reactions to—in human skin, 209.
- Galvanic stimulation, affecting pain  
 organs, 241.
- Giant capillaries, 100.
- Glomeruli, blood pressure in, 349;  
 capillary surfaces and volumes  
 in, 40, 349; capsular epithelium  
 of, 96; countings of—in kid-  
 neys, 41; filtration of protein  
 through, 362; filtration through,  
 348; opening and closing of, 249,  
 252, 254; structure of capillaries  
 in, 96.
- Gold salt, action on capillaries, 198.
- Graphite, suspensions for injection,  
 375; suspensions for perfusion,  
 380.
- Guanidin, capillary reactions to,  
 174.
- Gum, colloid osmotic pressure, 286,  
 367; in perfusion fluids, 187;  
 salts in, 367.
- Heart capillaries, distribution and  
 number, 29, 46.
- Heat, abnormal sensitivity to, 210;  
 effect of—on Bier's spots, 247;  
 increasing reactive hyperemia,  
 235; local vascular reactions to,  
 168, 209, 213.
- Hemorrhage, action of gum in, 367.
- Herpes Zoster, 118, 316.
- Histamine, action depending on  
 nerve supply, 206; action in  
 anesthesia, 196; action on frogs,  
 206; action on general circula-  
 tion, 57; action of human skin,  
 203; action on mammals, 205,  
 206; action on omentum, 203;  
 as a capillary poison, 200; at-  
 traction of leucocytes, 351; coun-  
 teracted by pituitrine, 194; on  
 arteries, 59; on pancreas, 60;  
 production in tissues, 207; shock,  
 202.
- Hormones (circulatory), adrena-  
 line, 194, 206; histamine, 207;  
 pituitary, 182, 194, 208.
- H-substance (*see also* dilator-sub-  
 stance), causing increased per-  
 meability, 334; from burnt tis-  
 sue, 357; in flare, 134; in reac-  
 tive hyperemia, 238; in the frog,  
 different from histamine, 239,  
 334; in wound shock, 356; liber-  
 ated in human skin by stimuli,  
 210; produced by dilator inner-  
 vation, 117, 239; produced in  
 urticaria factitia, 213; relation  
 to histamine, 213.
- Hydrogen ions, vascular reactions  
 to, 168; and capillary permea-  
 bility, 324.
- Hydrostatic pressure, counterbal-  
 anced by immersion, 305; in eye,  
 345; in thorax, 340; of blood in  
 veins, 298, 301.
- Hyperesthetic zones, reactions of  
 vessels in, 145, 148, 158.
- Hypersensitiveness, produced by  
 light, 216; produced by sugar in-  
 jection, 215.
- Hypophysis (*see also* pituitary),  
 extirpation in frogs, 182, 183;  
 structure, 183, 208.
- "Imbibition pressure," 291.
- Indian ink, impermeability of cap-  
 illaries to, 317; passage of—to  
 canal of Schlemm, 346; size of  
 particles, 317.
- Inflammation, 17, 21, 350; action  
 of pituitary extracts in, 194;  
 control of, 354; symptoms of,  
 350.
- Injection, methods for complete,  
 372; methods for vital, 373.

- Injury, increased permeability, 334; vascular response to, 258.
- Intestinal vessels (*see also* villi), reactions to skin stimulation, 148, 158; permeability of, 308.
- Itching, and pain, 134; as a symptom in flare, 132.
- Kupffer cells, structure and function of, 94.
- Leucocytes (*see* white corpuscles).
- Light, filters, 371; immunity to, 221; reactions to 215, 241.
- Liver capillaries, blood pressure in, 308; permeability of, 307; structure of, 94.
- Lymph, flow from intestine, 308; flow from liver, 308; flow from working organs, 312; protein content of—in frogs, 310; spaces and hearts of amphibia, 310; spaces of human skin, 99.
- Mechanical stimulation of capillaries, causing increased permeability, 333; direct reactions to, 163; in the human skin, 55; in the frog's tongue, 67, 125; initial dilatation caused by, 164.
- Metabolism, of muscle tissue, 23, 270; of skin, 36, 273; products responsible for reactive hyperemia, 235.
- Methylene blue, as vital stain for nerves, 108, 378; as vital stain for Rouget cells, 72, 74, 377.
- "Minute vessels," of human skin, 36; reactions in flare, 129.
- Mottling of skin, 263.
- Muscle, anatomy, 26; call for oxygen, 23.
- Muscle capillaries, diameters, 7, 29, 65; distribution and number, 26; during contraction, 27; numbers open during rest and work, 61, 257; surface of blood in, 30.
- Mustard gas, skin reactions to, 221.
- Mustard oil, acting mainly on nerves, 240; skin reactions to, 128.
- Myofibrils of smooth muscle, 76, 83, 90; staining of—with janus green, 377.
- Narcosis, action of—in wound shock, 356; action of—on capillaries, 195, 244; skin temperature in, 156.
- Naso-ocular reflex, 151.
- "Negative" pressure in thorax, mechanism of, 340.
- Nerves (*see also* sympathetic, dilator, nerve nets), medullated, 119; of capillaries, 108; path, determining localization of sensations, 142; vital staining of, 378.
- Nerve nets, decrement in, 125; degeneration, 110; formed by sympathetic fibers, 111, 115; of sensory fibers, 137, 141.
- Nervous system capillaries, development, 32; distribution and number, 31.
- Nettle poison, action of, 205, 351.
- Nictitating membrane, contraction of capillaries in, 50, 52.
- Occlusion (*see also* reactive hyperemia), affecting heat hyperemia, 214; arterial supply during, 246; causing white and red spots, 245; effect on H-substance, 211.
- "Oncoctic" pressure, 291.
- Osmotic pressure (*see also* colloid osmotic pressure), effective, 283, 323; of blood, 281; of colloids, 283; of sugar, 342.
- Oxygen, diffusion of—into frog's web, 190; diffusion of—in tissues, 270, 290; oxygen lack and capillary diameters, 228, 236, 274, 335; oxygen lack, capillary contractions during, 243; oxygen lack in relation to increased permeability, 321, 326, 335; oxygen

- lack produced by heat, 214; oxygen lack producing reactive hyperemia, 228, 235, 274; oxygen pressure in venous blood, 271; sensitivity of glomerular capillaries to lack of, 350; supply of—to muscles, 23, 271; supply of—to human skin, 273.
- Oxygen gland, rete mirabile of—in fishes, 42.
- Pain fibers, in flare, 134, 136; in inflammation, 350; in muscle arteries, 117; responsible for dilator innervation, 121.
- Pain receptors, sympathetic innervation of, 152.
- Paleness of death, 243, 256.
- Perfusion experiments, adrenaline in, 189; Hydrogen ions in, 168; methods, 379; oxygen in, 184; pituitrine in rhythmic, 380.
- Pericytes, 81.
- "Peripheral hearts," 48, 49.
- Peristalsis, of blood vessels in worms, 105; of capillaries, 19; of lymphatic capillaries, 105.
- Permeability, absolute capillary, 318, 326; changes in capillary, 315, 336; decrease in capillary, 338; differences in capillary, 307; effect of calcium on capillary, 336; effect of serum on capillary, 337; hormone, 338; of capillary wall, 266, 314; of capillary wall to colloids, 279; of capillary wall to crystalloids, 275; of frog's skin increased by veronal sodium, 178; test for proteins, 384.
- Pernicious anemia, red cells and hemoglobin in, 20.
- Phagocytosis, by endothelium, 95; by Kupffer cells, 95.
- Pigmentation, as a reaction to light, 216, 221; as a reaction to mustard gas, 222.
- Pituitary, action on chromatophores in frog, 182; extracts and permeability, 337; extracts, effects of, 184, 193; extracts in experimental inflammation, 194; extracts in histamine shock, 194; extracts, perfusion with, 184, 189; extracts, rôle of—in reactive hyperemia, 232; hormone, action on human skin, 192; hormone, concentration in blood, 191; hormone, for capillaries, 182; hormone, presence in circulating blood, 189.
- Plasma skimming (*see* blood flow).
- Plethora, causing edema, 333; opening of capillaries in, 256.
- Pleural cavity, absorption from, 340, 365; pressure in, 340.
- "Pressor" and "depressor" reactions, 57.
- Protein, breakdown of—prior to absorption, 365; content of frog's lymph, 310; in edema fluids, 361, 363; in urine, 361, 362; isoelectric point of, 283; molecular weight of, 284; osmotic pressure of, 283, 363; Spiegler's test for, 384.
- Psychic vascular reactions, 153.
- Pulse, action in stasis, 15; aortic regurgitation, influence on, 19, 102; in small vessels, 4, 7, 14, 19; in veins, 5, 102; method of measuring capillary pressure, 390; movements of arteries with, 4.
- Quantitative anatomy, 31, 46.
- Radium, skin reactions to, 221.
- Raynaud's gangrene, 263.
- Reactive hyperemia, 223; absence of—in intestine, 229; action on H-substance, 135; arm volume in, 224; blood flow in, 225; produced by cold, 180.
- Red corpuscles, agglutination, 13, 15; axial flow, 5; chloroform ac-

- tion on, 196; concentration of—  
in filtration edema, 360; count in  
capillary blood, 19; deformation,  
7; diapedesis, 15; distribution  
between arterial branches, 5;  
elasticity, 9; entrance into capil-  
laries from bone marrow, 16;  
fate after extrusion, 21; pack-  
ing, 14; transparency of packed,  
14.
- Reflected light, observations by, 3,  
61, 371; photography by, 372.
- Reflex (*see* axon reflex and spinal  
reflex).
- Reflex erythema (*see also* flare),  
123; in the frog's tongue, 123;  
in the frog's web, 126.
- "Refractoriness," of minute ves-  
sels, 331.
- Rete mirabile, in intestinal wall,  
345; in swim bladder, 42.
- Rouget cells, changes in appear-  
ance during contraction, 75; con-  
traction of single fibrils, 166;  
distribution, 91; history of, 72;  
in living animals, 80, 84; in  
worms, 105; myofibrils of, 90;  
nerve fibrils to, 110; nuclei of,  
77, 83, 84, 88, 98; on glomerular  
capillaries, 96; origin of, 94;  
possible syncytium, 162; rela-  
tion of—to adventitial cells, 82;  
response to tension, 166; stain-  
ing, 75; sympathetic innervation,  
80; tonus of, 90, 335.
- Scars, reactions of capillaries in,  
251.
- Secretion, of calcium in mammary  
gland, 275; through capillary  
wall, 266, 276, 342.
- Shifting of open capillaries, 253.
- Shock (circulatory), 354; action of  
crystalloids in, 367; action of  
gum and blood in, 368; an-  
aphylactic, 355; circulatory—  
produced by histamine, 202;  
toxemic—after burns, 356; toxe-  
mic—in peritonitis, 356.
- Skin color, affected by vessels of  
different size, 141; depending on  
blood in small vessels, 54; in  
Fundulus, 260.
- Skin temperature, affected by ar-  
terio-venous anastomoses, 104;  
determined by arterioles, 130.
- Skin vessels, distribution and num-  
ber in man, 32; in frog, perme-  
able to protein, 310; lymph  
spaces surrounding, 100; struc-  
ture, 97.
- Spinal reflex, from skin to intes-  
tinal vessels, 147; Lovén's, 150;  
Naso-ocular, 151; produced by  
cold, 151.
- Spleen, as blood volume regulator,  
265.
- Sprouting, of blood capillaries,  
21, 93; of lymphatic vessels, 21;  
of veins, 94.
- Stasis, 13, 14; filtration of plasma  
in, 315; in inflammation, 21; ir-  
reversibility, 15; mechanism,  
186; solution, 15; terminology,  
13, 21.
- Stigmatization, 120.
- Structure, in relation to size and  
function, 24, 306; of capillary  
wall, 70.
- Swim bladder (*see* oxygen gland).
- Sympathetic innervation, 111; axon  
reflexes, 143; causing paleness of  
death, 244; independent—on the  
two sides, 153; in vasoneurosis,  
262; long path axon reflexes,  
144; of capillaries, 53, 112; peri-  
arterial sympathectomy, 111;  
tonic, 113.
- Tar painting, vascular response to,  
260.
- Temperature regulation, denerva-  
tion and, 60; vascular mecha-  
nism, 154.



- Thebesian vessels, 46; anastomoses of—with arteries, 106.
- Tissue pressure, in the abdominal cavity, 304; in the skin, 304.
- Tone of capillaries, as a response to blood supply, 139; sympathetic, 113.
- Transmitted light, observation of circulation by, 3, 369.
- Triple response, after occlusion, 235, 238; produced by light, 218, 219; to histamine, 204; to indirect stimulation, 209.
- Urethane, action of, on frog's capillaries, 195, 334.
- Urticaria, 350; factitia, reactions in, 210; factitia, H-substance liberated in, 213; factitia, permeability of capillaries in, 331.
- Vasa serosa, 11.
- Vasoconstrictor substance, local production of, 247.
- Vasoneurosis, 260.
- Veins, blood pressure in, 301; blood pressure in—causing edema, 359; congestion of—producing reactive hyperemia, 229; contraction mechanism of liver veins, 264; determination of pressure in, 302; diminished tone in reactive hyperemia, 229; nests of small—in intestine, 344; spastic contraction of, 263; veno-pressure mechanism, 208; venous pump, 28, 303.
- Venules, in human skin, 100.
- Villi, capillary system of, 36; surface of, 38.
- Vital injection, of muscles, 63, 69, 114.
- Vitiligo areas, reaction to light, 221.
- Wheal, absorption of, 352; artificial, 330, 331, 352; composition of fluid in, 316; dye diffusion into, 330; filtration of plasma into, 316; not raised by renewed stimulation, 331; produced by histamine, 204; produced by injurious stimuli, 209; rate of production, 316; urticarial, 352.
- White corpuscles, adhesion to vessel walls, 17; chemotaxis, 18, 21, 351; circulation, 16; emigration, 17, 21, 351; specific gravity, 17.
- White reaction, absence of—in rabbit's ear, 164; delimitation, 141; in human skin, 55, 163; in internal organs, 165.
- X-rays, skin reactions to, 221.

# SILLIMAN MEMORIAL LECTURES

PUBLISHED BY YALE UNIVERSITY PRESS

**ELECTRICITY AND MATTER.** By Joseph John Thomson, D.Sc., LL.D., Ph.D., F.R.S., Fellow of Trinity College and Cavendish Professor of Experimental Physics, Cambridge University. (Fourth printing.)

**THE INTEGRATIVE ACTION OF THE NERVOUS SYSTEM.** By Charles S. Sherrington, D.Sc., M.D., Hon. LL.D. Tor., F.R.S., Holt Professor of Physiology, University of Liverpool. (Eighth printing.)

**EXPERIMENTAL AND THEORETICAL APPLICATIONS OF THERMODYNAMICS TO CHEMISTRY.** By Dr. Walter Nernst, Professor and Director of the Institute of Physical Chemistry in the University of Berlin.

**RADIOACTIVE TRANSFORMATIONS.** By Ernest Rutherford, D.Sc., LL.D., F.R.S., Macdonald Professor of Physics, McGill University. (Second printing.)

**THEORIES OF SOLUTIONS.** By Svante Arrhenius, Ph.D., Sc.D., M.D., Director of the Physico-Chemical Department of the Nobel Institute, Stockholm, Sweden. (Fourth printing.)

**IRRITABILITY.** A Physiological Analysis of the General Effect of Stimuli in Living Substances. By Max Verworn, M.D., Ph.D., Professor at Bonn Physiological Institute. (Second printing.)

**STELLAR MOTIONS.** With Special Reference to Motions Determined by Means of the Spectrograph. By William Wallace Campbell, Sc.D., LL.D., Director of the Lick Observatory, University of California. (Second printing.)

**PROBLEMS OF GENETICS.** By William Bateson, M.A., F.R.S., Director of the John Innes Horticultural Institution, Merton Park, Surrey, England. (Second printing.)

**THE PROBLEM OF VOLCANISM.** By Joseph Paxson Iddings, Ph.B., Sc.D. (Second printing.)

**PROBLEMS OF AMERICAN GEOLOGY.** By William North Rice, Frank D. Adams, Arthur P. Coleman, Charles D. Walcott, Waldemar Lindgren, Frederick Leslie Ransome and William D. Matthew. (Second printing.)

**ORGANISM AND ENVIRONMENT AS ILLUSTRATED BY THE PHYSIOLOGY OF BREATHING.** By John Scott Haldane, M.A., M.D., F.R.S., Hon. LL.D.

Birm. and Edin., Fellow of New College, Oxford; Honorary Professor, Birmingham University. (Second printing.)

**A CENTURY OF SCIENCE IN AMERICA.** With Special Reference to the American Journal of Science 1818-1918. By Edward Salisbury Dana, Charles Schuchert, Herbert E. Gregory, Joseph Barrell, George Otis Smith, Richard Swann Lull, Louis V. Pirsson, William E. Ford, R. B. Sosman, Horace L. Wells, Harry W. Foote, Leigh Page, Wesley R. Coe and George L. Goodale.

**A TREATISE ON THE TRANSFORMATION OF THE INTESTINAL FLORA WITH SPECIAL REFERENCE TO THE IMPLANTATION OF BACILLUS ACIDOPHILUS.** By Leo F. Rettger, Professor of Bacteriology, Yale University, and Harry A. Cheplin, Seessel Fellow in Bacteriology, Yale University.

**THE EVOLUTION OF MODERN MEDICINE.** By Sir William Osler, Bart., M.D., F.R.S. (Third printing.)

**RESPIRATION.** By J. S. Haldane, M.A., M.D., F.R.S., Hon. LL.D. Birm. and Edin., Fellow of New College, Oxford; Honorary Professor, Birmingham University.

**AFTER LIFE IN ROMAN PAGANISM.** By Franz Cumont. (Second printing.)

**THE ANATOMY AND PHYSIOLOGY OF CAPILLARIES.** By August Krogh, Ph.D., LL.D., Professor of Zoö-physiology, Copenhagen University. (Enlarged and revised edition.)

**LECTURES ON CAUCHY'S PROBLEM IN LINEAR PARTIAL DIFFERENTIAL EQUATIONS.** By Jacques Hadamard, LL.D., Member of the French Academy of Sciences; Foreign Honorary Member of the American Academy of Arts and Sciences.

**THE THEORY OF THE GENE.** By Thomas Hunt Morgan, LL.D., Sc.D., Ph.D., Professor of Biology, California Institute of Technology. (Enlarged and revised edition.)

**THE ANATOMY OF SCIENCE.** By Gilbert N. Lewis, Ph.D., Sc.D., Professor of Chemistry and Dean of the College of Chemistry, University of California. (Second printing.)

**BLOOD.** A Study in General Physiology. By Lawrence J. Henderson, A.B., M.D., Professor of Biological Chemistry in Harvard University.











3 2044 047 352 604

APR 02 2002 DATE DUE

Please Do Not Remove This Card from Pocket  
Please Do Not Remove This Card from Pocket

CALL (617) 432-2136 TO RENEW  
HAVE ID NUMBER & BOOK BARCODE  
or  
email to: [librenew@hms.harvard.edu](mailto:librenew@hms.harvard.edu)

DEMCO



3 2044 047 352 604